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(21) International Application Number: PCT/SE97/01217 (22) International Filing Date: 4 July 1997 (04.07.97) (30) Priority Data: 9602822-0 19 July 1996 (19.07.96) SE (71) Applicant (for all designated States except MG US): ASTRA PHARMA INC. [CA/CA]; 1004 Middlegate Road, Mississauga, Ontario L4Y 1M4 (CA). (71) Applicant (for MG only): ASTRA AKTIEBOLAG [SE/SE]; S-151 85 Södertälje (SE). (72) Inventors; and (75) Inventors/Applicants (for US only): SHI-HSIANG, Shen [CA/CA]; 161 Charwell Crescent, Beaconsfield, Quebec H9W 1C2 (CA). SULTAN, Ahmad [ID/CA]; 420 Bourke #1C, Dorval, Quebec H9S 3X1 (CA). WAHLESTEDT, Claes [SE/CA]; 6 Chelsea Place, Montreal, Quebec H3G 2J9 (CA). WALKER, Philippe [CH/CA]; 4551 Avenue de l'Esplanade, Montreal, Quebec H2T 2Y6 (CA). (74) Agent: ASTRA AKTIEBOLAG; Patent Dept., S-151 85 Södertälje (SE).		(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG). Published <i>With international search report.</i>
(54) Title: A NOVEL GALANIN RECEPTOR (57) Abstract The present invention is directed to a novel receptor for galanin which has been designated as galanin receptor 2. The invention encompasses both the receptor protein as well as nucleic acids encoding the protein. In addition, the present invention is directed to methods and compositions which rely upon either GAL-R2 proteins or nucleic acids.		

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A NOVEL GALANIN RECEPTOR

Field of the Invention

5 The present invention is in the general field of biological receptors and the various uses that can be made of such receptors. More specifically, the invention relates to nucleic acids encoding a novel galanin receptor and the receptor protein itself.

Background and Prior Art

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Galanin is a small (29-30 amino acid) neuroendocrine peptide which does not belong to any known peptide family (Bedecs *et al.*, *Int. J. Biochem. Cell. Biol.* 27: 337-349 (1995)). It is widely distributed in the central nervous system and other tissues, and has been reported to have a large number of diverse biological and pharmacological activities.

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Galanin has been reported to: (a) promote growth hormone release (Bauer *et al.*, *The Lancet* 2:192-195 (1986)); (b) inhibit glucose-induced insulin release (Ahren *et al.*, *FEBS Lett.* 299:233-237 (1988)); (c) regulate motility in the gastrointestinal tract (Fox-Thelkeld *et al.*, *Gastroenterology* 101:1471-1476 (1991)); (d) stimulate feeding behavior (Crawley *et al.*, *J. Neurosci* 10:3695-3700 (1990)); and (e) impair cognitive function (Mastropaolo *et al.*, *Proc. Nat'l Acad. Sci. U.S.A.* 85:9841-9845 (1988)).

20

Of particular pharmacological interest are galanin's analgesic effects (Post *et al.*, *Acta Physiol. Scand.* 132:583-584 (1988)). In the spinal cord, galanin inhibits nociceptive reflexes and potentiates the analgesic effect of morphine (Wiesenfeld-Hallin *et al.*, *Neurosci. Lett.* 105:149-154 (1989)). Target administration of galanin hyperpolarizes dorsal horn neurons and chronic administration of a galanin receptor antagonist after axotomy has been reported to markedly increase autonomy in rats (Verge *et al.*, *Neurosci. Lett.* 149:193-197 (1993)). These observations indicate that galanin, like morphine, has strong anti-nociceptive actions *in vivo*. Thus, the known pharmacological effects of galanin suggest potential therapeutic applications as an anesthetic or analgesic in animals and humans.

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Galanin exerts its effects by binding to membrane-bound receptors. The cDNA for one such receptor ("GAL-R1") has been cloned from both humans and rats (Habert-Ortoliet *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 91:9780-9783 (1994); Burgevin *et al.*, *J. Mol. Neurosci.* 6:33-41 (1995)). High levels of rat GAL-R1 mRNA have been found in the ventral

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hippocampus, thalamus, amygdala, and medulla oblongata of the brain and in the dorsal horn of the spinal cord (Burgevin *et al.*, *supra*). Pharmacological data obtained using galanin fragments, agonists and antagonists have suggested that more than one type of receptor may be responsible for galanin's actions (for a review, see Valkna *et al.*, *Neurosci. Lett.* 187:75-78 (1995)). The isolation and characterization of new receptors for galanin would be highly desirable to assist in the discovery and development of therapeutic agents for altering galanin activity *in vivo*.

Summary of the Invention

The present invention is based upon the discovery of a novel galanin receptor ("GAL-R2") which is distinct from previously reported receptors in terms of structure, tissue distribution and binding characteristics. Receptors from both the rat and human have been isolated and sequenced. As used herein, the term "GAL-R2" refers to the receptor from either of these species unless the text, expressly or by context, indicates otherwise.

In its first aspect, the invention is directed to proteins, except as existing in nature, comprising the amino acid sequence consisting functionally of rat GAL-R2 (as shown in figure 1) or consisting functionally of human GAL-R2 (as shown in figure 2). The term "consisting functionally of" refers to proteins in which the sequence of figure 1 or figure 2 has undergone additions, deletions or substitutions which do not substantially alter the functional characteristics of the receptor. Thus, the invention encompasses proteins having exactly the same amino acid sequence as shown in the figures, as well as proteins with differences that are not substantial as evidenced by their retaining the basic, qualitative ligand binding properties of GAL-R2. The invention further encompasses substantially pure proteins consisting essentially of a GAL-R2 amino acid sequence, antibodies that bind specifically to GAL-R2 (i.e. that have at least a 100 fold greater affinity for GAL-R2 than any other protein), and antibodies made by a process involving the injection of pharmaceutically acceptable preparations of such proteins into an animal capable of antibody production. In a preferred embodiment, monoclonal antibody to GAL-R2 is produced by injecting the pharmaceutically acceptable preparation of GAL-R2 into a mouse and then fusing mouse spleen cells with myeloma cells.

The invention is also directed to a substantially pure polynucleotide encoding a protein comprising the amino acid sequence consisting functionally of the sequence of rat GAL-R2 (as shown in figure 1) or human GAL-R2 (as shown in figure 2). This aspect of the

invention encompasses polynucleotides encoding proteins consisting essentially of the amino acid sequences of in the figures, expression vectors comprising such polynucleotides, and host cells transformed with such vectors. Also included is the recombinant rat and human GAL-R2 proteins produced by host cells made in this manner.

5 Preferably, the polynucleotide encoding rat GAL-R2 has the nucleotide sequence shown in figure 1 and the polynucleotide encoding human GAL-R2 has the nucleotide sequence shown in figure 2. It is also preferred that the vectors and host cells used for the expression of GAL-R2 use these particular polynucleotides.

10 In another aspect, the present invention is directed to a method for assaying a test compound for its ability to bind to GAL-R2. This method is performed by incubating a source of GAL-R2 with a ligand known to bind to the receptor and with the test compound. The source of GAL-R2 should be substantially free of other types of galanin receptors, i.e. greater than 90% of the galanin receptors present should correspond to GAL-R2. Upon

15 completion of incubation, the ability of the test compound to bind to GAL-R2 is determined by the extent to which ligand binding has been displaced. A preferred source of GAL-R2 for use in the assay is a cell transformed with a vector for expressing the receptor and comprising a polynucleotide encoding a protein consisting essentially of the amino acid sequence shown in figure 1(rat GAL-R2) and figure 2 (human GAL-R2).

20 Instead of using cells in the assay, a membrane preparation can be prepared from the cells and this can be used as the source of GAL-R2. Although not essential, the assay can be accompanied by the determination of the activation of a second messenger pathway such as the adenylyl cyclase pathway. This should help to determine whether a compound that binds to GAL-R2 is acting as an agonist or antagonist to galanin.

25 In another aspect, the present invention is directed to a method for assaying a test compound for its ability to alter the expression of GAL-R2. This method is performed by growing cells expressing GAL-R2, but substantially free of other galanin receptors, in the presence of the test compound. Cells are then collected and the expression of GAL-R2 is

30 compared with expression in control cells grown under essentially identical conditions but in the absence of the test compound. In preferred embodiments, the cells expressing GAL-R2 are cells transformed with an expression vector comprising a polynucleotide sequence encoding a protein consisting essentially of the amino acid sequence shown in figure 1 (rat GAL-R2) or figure 2 (human GAL-R2). A preferred test compound is an oligonucleotide at

35 least 15 nucleotides in length and comprising a sequence complementary to a sequence

shown in one or both of the figures. The preferred method for determining receptor expression is by means of a receptor binding assay.

Brief Description of the Drawings

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Figure 1: The composite nucleotide sequence and corresponding translated amino acid sequence (in single letter code) of rat GAL-R2 is shown. The nucleic acid sequence has been given the designation SEQ ID NO:1 and the amino acid sequence, SEQ ID NO:2.

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Figure 2: The composite nucleotide sequence and corresponding translated amino acid sequence (in single letter code) of human GAL-R2 is shown. The nucleic acid sequence has been given the designation SEQ ID NO:3 and the amino acid sequence, SEQ ID NO:4.

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Figure 3: The amino acid sequences of rat GAL-R2 (RGALR2.PRO), rat GAL-R1 (rGALR1.PRO), human GAL-R2(HGALR2.PRO) and human GAL-R1 (hGALR1.PRO) are aligned to show regions of homology. The residues in the HGAL-R2 sequence that are shared with other sequences are boxed. In order to optimize alignment, gaps were created at several places in GAL-R2 sequences and these gaps are indicated by black boxes. The rGALR1.PRO sequence has been designated as SEQ ID NO:5; and the hGALR1.PRO

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Figure 4: The saturation isotherm of ^{125}I -galanin binding to membranes from rat GAL-R2-expressing HEK-293 cells is shown. Increasing concentrations of radiotracer were incubated with the membranes, binding was allowed to reach equilibrium, and then the reaction was filtered as described under Example 3. Nonspecific binding was measured in the presence of 1 μM of unlabeled galanin and was subtracted from total binding to obtain specific binding.

25

Figure 5: Figure 4 shows the results of binding assays in which unlabeled galanin and galanin-related peptides were allowed to compete with labeled galanin for GAL-R2 sites. The data has been converted to percentages, with binding in the absence of competitor serving as 100%. No inhibition was observed when binding assays were performed in the presence of peptides unrelated to galanin.

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Figure 6: Galanin attenuated the stimulation of adenylyl cyclase by forskolin in a dose-dependent manner in HEK-293 cells expressing GAL-R2. Panel A shows the basal level of

cAMP in cells not treated with either forskolin or galanin (C); the effect of 1 μ M galanin (G); the effect of 0.1 mM forskolin (F); and the effect of 1 μ M galanin + 0.1 mM forskolin (F+G). In panel B, cells were incubated in the presence of 0.1 mM forskolin alone or in the presence of forskolin with various concentrations of galanin. Intracellular cAMP was then
5 extracted and measured by enzyme immunoassay as described in Example 4. Results are expressed as percentages, where 100% is the value obtained in the presence of forskolin alone.

Definitions

10

The description that follows uses a number of terms that refer to recombinant DNA technology. In order to provide a clear and consistent understanding of the specification and claims, including the scope to be given such terms, the following definitions are provided.

15

Cloning vector: A plasmid or phage DNA or other DNA sequence which is able to replicate autonomously in a host cell, and which is characterized by one or a small number of restriction endonuclease recognition sites. A foreign DNA fragment may be spliced into the vector at these sites in order to bring about the replication and cloning of the fragment.
20 The vector may contain a marker suitable for use in the identification of transformed cells. For example, markers may provide tetracycline resistance or ampicillin resistance.

25

Expression vector: A vector similar to a cloning vector but which is capable of inducing the expression of the DNA that has been cloned into it, after transformation into a host.
The cloned DNA is usually placed under the control of (i.e., operably linked to) certain regulatory sequences such as promoters or enhancers. Promoter sequences may be constitutive, inducible or repressible.

30

Substantially pure: As used herein, "substantially pure" means that the desired product is essentially free from contaminating cellular components. Contaminants may include, but are not limited to, proteins, carbohydrates or lipids. One method for determining the purity of a protein or nucleic acid is by electrophoresing a preparation in a matrix such as polyacrylamide or agarose. Purity is evidenced by the appearance of a single band after staining.

35

Host: Any prokaryotic or eukaryotic cell that is the recipient of a replicable expression vector or cloning vector is the "host" for that vector. The term encompasses prokaryotic or eukaryotic cells that have been engineered to incorporate a desired gene on its chromosome or in its genome. Examples of cells that can serve as hosts are well known in the art, as are techniques for cellular transformation (see e.g. Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor (1989)).

Promoter: A DNA sequence typically found in the 5' region of a gene, located proximal to the start codon. Transcription is initiated at the promoter. If the promoter is of the inducible type, then the rate of transcription increases in response to an inducing agent.

Complementary Nucleotide Sequence: A complementary nucleotide sequence, as used herein, refers to the sequence that would arise by normal base pairing. For example, the nucleotide sequence 5'-AGAC-3' would have the complementary sequence 5'-GTCT-3'.

Expression: Expression is the process by which a polypeptide is produced from DNA. The process involves the transcription of the gene into mRNA and the translation of this mRNA into a polypeptide.

Detailed Description of the Invention

The present invention is directed to the GAL-R2 receptor proteins, genetic sequences coding for the receptors, a method for assaying compounds for binding to GAL-R2 and a method for assaying compounds for their ability to alter GAL-R2 expression. The receptors and their nucleic acids are defined by their structures (as shown in figures 1 and 2) as well as by their tissue distribution and binding characteristics.

With respect to structure, it will be understood that the present invention encompasses not only sequences identical to those shown in the figures, but also sequences that are essentially the same and sequences that are otherwise substantially the same and which result in a receptor retaining the basic binding characteristics of GAL-R2. For example, it is well known that techniques such as site-directed mutagenesis may be used to introduce variations in a protein's structure. Variations in GAL-R2 introduced by this or some similar method are encompassed by the invention provided that the resulting receptor retains the ability to specifically bind to galanin or galanin-like peptides. Thus, the invention relates to

proteins comprising amino acid sequences consisting functionally of the sequence of SEQ ID NO:2 (rat) and SEQ ID NO:4 (human).

I. Nucleic Acid Sequences Coding for GAL-R2

5

DNA sequences coding for GAL-R2 are present in a variety of tissues, any of which may serve as a source for the isolation of nucleic acid coding for the receptor. In rats, spinal cord and brain tissues are among the preferred sources with the dorsal ganglia of the spinal cord and the hippocampus, mammillary bodies and cerebellum of the brain being especially preferred. In addition, cells and cell lines that express GAL-R2 may serve as a source for nucleic acid. These may either be cultured cells that have not undergone transformation or cell lines specifically engineered to express recombinant GAL-R2.

10

Many methods are available for isolating DNA sequences and may be adapted for the isolation of GAL-R2 nucleic acid (see for example Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Press (1989)). One preferred method for rat GAL-R2, illustrated in Example 1, is to screen a cDNA library that has been prepared by reverse transcribing mRNA isolated from tissues or cells known to express GAL-R2. The library may be prepared from, for example, rat dorsal root ganglia or from brain tissue. A rat brain stem spinal cord cDNA library in ZAP II has been found to produce suitable results. A similar method can be used for human GAL-R2 or, alternatively, a human DNA library can be screened as described in Example 7.

15

It is expected that a wide variety of probes specific for GAL-R2 can be used equally well for the screening of cDNA libraries. One way to easily produce a large amount of probe is to use the polymerase chain reaction (PCR) to amplify the desired sequence from a cDNA library. For example, PCR may be performed on a cDNA library from rat dorsal root ganglia using the primers:

25

TM2: 5'-GGCCGTCGACTTCATCGTC(AorT)(AorC)(TorC)CTI(GorT)
CI(TorC)TIGC(A,C,GorT)GAC-3' (SEQ ID NO:7)

30

TM7: 5'-(AorG)(C,AorT)(AorT)(AorG)CA(AorG)TAIATATIGG(AorG)TT-3'
(SEQ ID NO:8)

35

The letter "I" in the sequences above, is the abbreviation for inosine.

Amplified fragments can be size fractionated on an agarose gel and the selected fragments (e.g., fragments 400-1,000 base pairs in length) inserted into an appropriate vector (e.g., pGEM-T). The vector may be introduced into competent cells (e.g., DH5 cells) by any of the established methods for cell transformation, e.g., by calcium phosphate precipitation.

5 Transformed cells containing the DNA of interest may be identified by again performing PCR with the TM2 and TM7 primers. The DNA inserts present in these cells are excised, purified and labeled with ^{32}P . The labeled DNA fragments thus produced are used as probes for screening a cDNA library for GAL-R2. The presence of the correct sequence in selected cells may be confirmed by DNA sequencing and, if necessary, partial clones may

10 be spliced together to form a full-length sequence.

Although the above procedure is known to be suitable for obtaining GAL-R2 nucleic acid, it is expected that alternative techniques can be developed with relatively little effort. Thus, cDNA libraries may be screened using probes synthesized based upon the GAL-R2

15 sequence shown in figure 1 for rats and shown in figure 2 for humans. In general, probes should be at least 14 nucleotides long and should not be selected from regions known to be highly conserved among proteins, e.g., the transmembrane domains of G-protein linked receptors. Alternatively, using the sequences shown in the figures, it should be possible to select PCR primers that amplify the full-length GAL-R2 sequence. The same techniques

20 that have proven successful in the rat and human can be used to obtain GAL-R2 sequences from other species as well.

II. Production and Isolation of GAL-R2 Recombinant Protein

25 In order to express recombinant GAL-R2, the structural sequence for the protein described above must be placed in a vector containing transcriptional and translational signals recognizable by an appropriate host. The cloned GAL-R2 sequences, preferably in double-stranded form, are inserted into the expression vector in an operable linkage, i.e., they are

30 positioned so as to be under the control of the vector's regulatory sequences and in such a manner that mRNA is produced which is translated into the GAL-R2 amino acid sequence.

Expression of the GAL-R2 receptor protein in different hosts may result in different post-translational modifications that can, potentially, alter the properties of the receptor.

35 Preferably, nucleic acid encoding GAL-R2 is expressed in eukaryotic cells, especially mammalian cells. These cells provide post-translational modifications which, *inter alia*, aid

in the correct folding of the receptor protein. Examples of an appropriate vector, pCDNA3-GAL-R2, and host, HEK293 cells, are given in the Example 2.

Other mammalian cells that may be used include, without limitation, NIH-3T3 cells, CHO cells, HeLa cells, LM(tk-) cells etc. Vectors suitable for use in each of these various cell types are well known in the art (see e.g. Sambrook *et al.*, *supra*). Preferred eukaryotic promoters include that of the mouse metallothionein I gene; the TK promoter of Herpes virus; the SV40 early promoter; and the yeast GAL4 gene promoter. Some examples of suitable prokaryotic promoters include those capable of recognizing T4 polymerases, the P_R and P_L promoters of bacteriophage lambda, and the trp, recA, heat shock and lacZ promoters of *E. coli*.

Expression vectors may be introduced into host cells by methods such as calcium phosphate precipitation, microinjection or electroporation. Cells expressing the GAL-R2 receptor can be selected using methods well known in the art. One simple method for confirming the presence of the receptor nucleic acid in cells is to perform PCR amplification using the procedures and primers discussed above. The presence of functional receptor may be confirmed by performing binding assays using labeled galanin.

Once cells producing recombinant GAL-R2 receptor have been identified, they may be used in either binding assays or in assays designed to identify agents capable of altering GAL-R2 expression. Alternatively, membranes may be isolated from the cells and used in receptor binding assays.

III. Antibodies to GAL-R2

The present invention also is directed to antibodies that bind specifically to GAL-R2 and to a process for producing such antibodies. Antibodies that "bind specifically to GAL-R2" are defined as those that have at least a one hundred fold greater affinity for GAL-R2 than for GAL-R1 and any undenatured protein not binding galanin. The process for producing such antibodies may involve either injecting the GAL-R2 protein itself into an appropriate animal or, preferably, injecting short peptides made to correspond to different regions of GAL-R2. The peptides should be at least five amino acids in length and should be selected from regions believed to be unique to the GAL-R2 protein. Thus, highly conserved transmembrane regions should generally be avoided in selecting peptides for the generation

of antibodies. Methods for making and detecting antibodies are well known to those of skill in the art as evidenced by standard reference works such as: Harlow *et al.*, *Antibodies, A Laboratory Manual*, Cold Spring Harbor Laboratory, N.Y. (1988)); Klein, *Immunology: The Science of Self-Nonself Discrimination* (1982); Kennett, *et al.*, *Monoclonal Antibodies and Hybridomas: A New Dimension in Biological Analyses* (1980); and Campbell, "Monoclonal Antibody Technology," in *Laboratory Techniques in Biochemistry and Molecular Biology*, (1984)).

"Antibody," as used herein, is meant to include intact molecules as well as fragments which retain their ability to bind to antigen (e.g., Fab and F(ab)₂ fragments). These fragments are typically produced by proteolytically cleaving intact antibodies using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab)₂ fragments). The term "antibody" also refers to both monoclonal antibodies and polyclonal antibodies. Polyclonal antibodies are derived from the sera of animals immunized with the antigen. Monoclonal antibodies can be prepared using hybridoma technology (Kohler, *et al.*, *Nature* 256:495 (1975); Hammerling, *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas*, Elsevier, M.Y., pp. 563-681 (1981)). In general, this technology involves immunizing an animal, usually a mouse, with either intact GAL-R2 or a fragment derived from GAL-R2. The splenocytes of the immunized animals are extracted and fused with suitable myeloma cells, e.g., SP₂O cells. After fusion, the resulting hybridoma cells are selectively maintained in HAT medium and then cloned by limiting dilution (Wands, *et al.*, *Gastroenterology* 80:225-232 (1981)). The cells obtained through such selection are then assayed to identify clones which secrete antibodies capable of binding to GAL-R2.

The antibodies, or fragments of antibodies, of the present invention may be used to detect the presence of GAL-R2 protein using any of a variety of immunoassays. For example, the antibodies may be used in radioimmunoassays or in immunometric assays, also known as "two-site" or "sandwich" assays (see Chard, T., "An Introduction to Radioimmune Assay and Related Techniques," in *Laboratory Techniques in Biochemistry and Molecular Biology*, North Holland Publishing Co., N.Y. (1978)). In a typical immunometric assay, a quantity of unlabelled antibody is bound to a solid support that is insoluble in the fluid being tested, e.g., blood, lymph, cellular extracts, etc. After the initial binding of antigen to immobilized antibody, a quantity of detectably labeled second antibody (which may or may not be the same as the first) is added to permit detection and/or quantitation of bound antigen (see e.g. *Radioimmune Assay Method*, Kirkham *et al.*, e.d., pp. 199-206, E & S.

Livingstone, Edinburgh (1970)). Many variations of these types of assays are known in the art and may be employed for the detection of GAL-R2.

Antibodies to GAL-R2 may also be used in the purification of either the intact receptor or
5 fragments of the receptor (see generally, Dean *et al.*, Affinity Chromatography, A Practical Approach, IRL Press (1986)). Typically, antibody is immobilized on a chromatographic matrix such as Sepharose 4B. The matrix is then packed into a column and the preparation containing GAL-R2 is passed through under conditions that promote binding, e.g., under
10 conditions of low salt. The column is then washed and bound GAL-R2 is eluted using a buffer that promotes dissociation from antibody, e.g., buffer having an altered pH or salt concentration. The eluted GAL-R2 may be transferred into a buffer of choice, e.g., by dialysis, and either stored or used directly.

15 IV. Assay for GAL-R2 Binding

One of the main uses for GAL-R2 nucleic acids and recombinant proteins is in assays designed to identify agents, other than galanin, capable of binding to GAL-R2 receptors. Such agents may either be agonists, mimicking the effects of galanin, or antagonists,
20 inhibiting the effects of galanin. Of particular interest is the identification of agents which bind to the GAL-R2 receptors and modulate adenylyl cyclase activity in the cells. These agents have potential therapeutic application as either analgesics or anesthetics.

An example of an assay that may be used for detecting compounds binding to GAL-R2 is
25 presented in Example 4. The essential feature of this assay is that a source of GAL-R2 is incubated together with a ligand known to bind to the receptor and with the compound being tested for binding activity. The preferred source for GAL-R2 is cells, preferably mammalian cells, transformed to recombinantly express the receptor. The cells selected should not express a substantial amount of any other receptor which binds galanin, e.g.,
30 GAL-R1. This can easily be determined by performing galanin binding assays on cells derived from the same tissue or cell line as those recombinantly expressing GAL-R2 but which have not undergone transformation.

The assay may be performed either with intact cells or, preferably, with membranes
35 prepared from the cells (see e.g. Wang, *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 90:10230-10234 (1993)). The membranes are incubated with a ligand specific for galanin receptors

and with a preparation of the compound being tested. After binding is complete, receptor is separated from the solution containing ligand and test compound, e.g. by filtration, and the amount of binding that has occurred is determined. Preferably, the ligand used is galanin detectably labeled with a radioisotope such as ^{125}I . However, if desired, fluorescent or chemiluminescent labels can be used instead. Among the most commonly used fluorescent labeling compounds are fluorescein isothiocyanate, rhodamine, phycoerythrin, phycocyanin, allophycocyanin, *o*-phthaldehyde and fluorescamine. Useful chemiluminescent compounds include luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt, and oxalate ester. Any of these agents which can be used to detectably label galanin will produce a ligand suitable for use in the assay.

Nonspecific binding may be determined by carrying out the binding reaction in the presence of a large excess of unlabelled ligand. For example, ^{125}I -galanin may be incubated with receptor and test compound in the presence of a thousandfold excess of unlabelled galanin. Nonspecific binding should be subtracted from total binding, i.e. binding in the absence of unlabeled galanin, to arrive at the specific binding for each sample tested. Other steps such as washing, stirring, shaking, filtering and the like may be included in the assays as necessary. Typically, wash steps are included after the separation of membrane-bound ligand from ligand remaining in solution and prior to quantitation of the amount of ligand bound, e.g., by counting radioactive isotope. The specific binding obtained in the presence of test compound is compared with that obtained in the presence of labeled ligand alone to determine the extent to which the test compound has displaced galanin.

In performing binding assays, care must be taken to avoid artifacts which may make it appear that a test compound is interacting with the GAL-R2 receptor when, in fact, binding is being inhibited by some other mechanism. For example, the compound being tested should be in a buffer which does not itself substantially inhibit the binding of galanin to GAL-R2 and should, preferably, be tested at several different concentrations. Preparations of test compound should also be examined for proteolytic activity and it is desirable that antiproteases be included in assays. Finally, it is highly desirable that compounds identified as displacing the binding of ligand to GAL-R2 receptor be reexamined in a concentration range sufficient to perform a Scatchard analysis on the results. This type of analysis is well known in the art and can be used for determining the affinity of a test compounds for receptor (see e.g., Ausubel, *et al.*, Current Protocols in Molecular Biology, 11.2.1-11.2.19 (1993); Laboratory Techniques and Biochemistry and Molecular Biology,

Work, *et al.*, ed., N.Y. (1978) etc.). Computer programs may be used to help in the analysis of results (see e.g., Munson, P., *Methods Enzymol.* 92:543-577 (1983); McPherson, G.A., *Kinetic, EBDA Ligand, Lowry-A Collection of Radioligand Binding Analysis Programs*, Elsevier-Biosoft, U.K. (1985)). An example of the types of curves that may be obtained using this method is shown in figure 5 and examples of inhibitory constants for galanin-related peptides determined using binding assays are shown in Table 1.

The activation of a second messenger pathway may be examined by performing adenylyl cyclase assays for compounds that have been identified as binding to the GAL-R2 receptor. These assays may be carried out as discussed in Example 5 or using any other method for determining cAMP concentration. Typically, adenylyl cyclase assays will be performed separately from binding assays, but it may also be possible to perform binding and adenylyl cyclase assays on a single preparation of cells.

V. Assay for Ability to Modulate GAL-R2 Expression

One way to either increase or decrease the biological effects of galanin is to alter the extent to which GAL-R2 is expressed in cells. Therefore, assays for the identification of compounds that either inhibit or enhance expression are of considerable interest. These assays are carried out by growing cells expressing GAL-R2 in the presence of a test compound and then comparing receptor expression in these cells with cells grown under essentially identical conditions but in the absence of the test compound. As in the binding assays discussed above, it is desirable that the cells used be substantially free of receptors for galanin other than GAL-R2. Scatchard analysis of binding assays performed with labeled galanin can be used to determine receptor number. The binding assays may be carried out as discussed above in section IV and will preferably utilize cells that have been engineered to recombinantly express GAL-R2 as described in sections I and II.

A preferred group of test compounds for inclusion in the GAL-R2 expression assay consists of oligonucleotides complementary to various segments of the GAL-R2 nucleic acid sequence. These oligonucleotides should be at least 15 bases in length and should be derived from non-conserved regions of the receptor nucleic acid sequence.

Oligonucleotides which are found to reduce receptor expression may be derivatized or conjugated in order to increase their effectiveness. For example, nucleoside phosphoro-

thioates may be substituted for their natural counterparts (see Cohen, J., Oligodeoxynucleotides, Antisense Inhibitors of Gene Expression, CRC Press (1989)). The oligonucleotides may be delivered to a patient *in vivo* for the purpose of inhibiting GAL-R2 expression. When this is done, it is preferred that the oligonucleotide be administered in a form that enhances its uptake by cells. For example, the oligonucleotide may be delivered by means of a liposome or conjugated to a peptide that is ingested by cells (see e.g., U.S. Patent Nos. 4,897,355 and 4,394,448; see also non-U.S. patent documents WO 8903849 and EP 0263740). Other methods for enhancing the efficiency of oligonucleotide delivery are well known in the art and are also compatible with the present invention.

Having now described the invention, the same will be more readily understood through reference to the following Examples which are provided by way of illustration and which are not intended to limit the scope of the invention.

EXAMPLES

Example 1: Cloning of Rat Galanin Receptor-2 (GAL-R2)

A PCR-based homology screening strategy was used to isolate novel cDNA sequences encoding G protein-coupled receptors. Sequences likely to encode G protein-coupled receptors were amplified from rat dorsal root ganglia mRNA by reverse transcription PCR using the following primers:

TM2: 5'-GGCCGTCGACTTCATCGTC (A or T)(A or C)(T or C)CT
I (G or T)C I (T or C)T I GC(A,C,G or T) GAC -3' (SEQ ID NO:5)

TM7: 5'-(A or G)(C,A or T)(A or T)(A or G)CA (A or G)TAIAT
IATIGG(A or G)TT -3' (SEQ ID NO:6)

The templates for PCR amplification were synthesized using a "First Strand cDNA Synthesis Kit" (Pharmacia Biotech) and 400 ng of dorsal root ganglia poly A+ RNA. The first strand cDNA thus prepared was diluted two fold with distilled water, heated at 95 C for 3 minutes and quickly chilled on ice. 5 µL of the cDNA thus produced was then

amplified with 50 pmoles of each of the TM2 and TM7 primers and 2.5 units of Taq DNA polymerase in 50 mM KCl, 1.5 mM MgCl₂, 10 mM Tris(HCl), and 200 μM dNTPs, pH 9.0. The reaction tubes were heated at 95°C for one min. and then subjected to 40 cycles of denaturation (95°C/1 min), annealing (45°C/1 min) and extension (72°C/1 min). The final extension was performed for 10 min. The amplified fragments were analyzed and size fractionated on a 1.5% agarose gel. Fragments between 400 bp and 1000 bp in length were excised from the gel, purified using a Sephaglas BandPrep kit from Pharmacia, and inserted into a pGEM-T vector from Promega. The recombinant plasmids thus produced were used to transform competent DH5 cells.

Transformed cells were plated on ampicilline-containing 2YT agar plates and recombinant pGEM-T clones were selected by direct colony PCR using primers designed for T7 and SP6 promoters. The PCR conditions were exactly the same as above except 50 pmole each of T7 and SP6 primers were used instead of TM2 and TM7 primers. The annealing temperature was 50 C and 30 cycles were performed. Plasmid DNA was prepared from the clones containing recombinant plasmids using a "Wizard Miniprep DNA Purification System" (Promega Corporation) starting with 4 ml of bacterial culture. The DNA sequence from these clones was determined using the Sanger dideoxynucleotide chain termination method on denatured double-stranded plasmid templates using a T7 sequencing kit from Pharmacia.

The insert DNA fragment of clone 3B-21 was excised from the vector using Sac II and Nde I, isolated on an agarose gel and labeled with ³²P by random primed synthesis using a Ready-To-Go DNA labeling kit from Pharmacia. This labeled fragment was used to screen a rat brain stem spinal cord cDNA library in ZAP II (Stratagene). Filters were prehybridized for 2 hours at 42°C in 50% formamide, 5x SSC, 5x Denhardt's solution, 1% glycine and 100 μg/ml denatured and sheared salmon sperm DNA. Hybridization with labeled probe was performed at 42 C for 18 hours in a solution containing 50% formamide, 5X SSC, 1x Denhardt's solution, 0.3% SDS and 100 μg/ml denatured and sheared salmon sperm DNA. Filters were rinsed twice in 2x SSC, 0.1% SDS at room temperature. They were then washed twice for 15 min in 2x SSC, 0.1% SDS at 42 C, twice for 15 min at 42 C in 0.2x SSC, 0.1% SDS, twice with 0.05x SSC, 0.1% SDS at 55 C and finally in the same wash solution at 65 C.

Hybridization-positive phages were purified and their inserts rescued by helper phage mediated excision to yield plasmid DNAs. One clone, 21RSC4, contained the complete

coding sequence for the receptor except for 51 bp of the 5' region. This region was obtained by PCR and was then joined at the Bsu36I site at nucleotide number 16 of 21RSC4. Thus, 67 bp at the 5'-end of the coding region of the clone pBS/GALR-2 arose from a PCR-generated fragment.

Example 2: Structural Characteristics of Rat Galanin Receptor-2

The recombinant plasmid pBS/GALR-2 was found to contain an open reading frame of 372 amino acids, flanked by 3' and 5' untranslated regions of, respectively, 289 and 308 bp. The sequence of the open reading frame is shown in figure 1 along with the amino acid sequence of the encoded protein. The protein has a molecular mass of 40,700 daltons. Hydropathy analysis of the protein is consistent with a topography of seven transmembrane domains, indicative of the G-protein-coupled receptor family (Sprengel *et al.*, "Hormone Receptors," in Handbook of Receptors and Channels: G Protein-Coupled Receptors, Peroutka, S.J., ed., pp. 153-207, CRC Press (1994)). In addition, sequence analysis revealed that the open reading frame of pBS/GALR-2 contains several conserved structural features/residues found among the members of the neuropeptide receptor family, including: an asparagine in TM1 (Asn43); a leucine (Leu67) and an aspartic acid (Asp 71) in TM2; and an arginine (Arg123) and Tyrosine residue (Tyr124) in TM3. Other features of this GAL-R2 receptor gene are: potential sites for N-glycosylation in the amino terminus (Asn2, Asn11); the presence of several serines and threonines in the carboxyl terminus; and the presence of a second and third intracellular loop, which may serve as potential sites for phosphorylation by protein kinases.

A comparison of the rat GAL-R2 open reading frame with the sequences of human GAL-R2 and GAL-R1 receptors is shown in figure 3. Overall, rat GAL-R2 has an identity of about 53 % at the nucleotide level and 35.5% at the amino acid level with rat GAL-R1 (Burgevin *et al.*, *J. Mol. Neurosci.* 6:33-41 (1995)) and 34.8% with human GAL-R1 (Habert-Ortoli *et al.*, *Proc. Natl. Acad. Sci. USA* 91:9780-9783 (1994)). However the sequence homology is higher in the putative transmembrane domains. Respectively, the homologies between the known rat GAL-R1 and GAL-R2 in TM1 to TM7 are 37.5%, 67%, 41.6%, 25%, 50%, 33% and 50%.

Overall, it is apparent that GAL-R2 has a unique sequence that sets it apart from the other G-protein-coupled receptors or other members of the neuropeptide receptor subfamily. The

amino acid residues essential for the binding of galanin to the GAL-R1 receptor have been identified as His264, His267, Phe282 and, to a lesser extent, Glu271. Only one of these residues, corresponding to His264, is conserved in GAL-R2.

5

Example 3: Recombinant Expression of Rat Galanin Receptor-2

To generate a mammalian expression vector, a 1.4 Kb Hind III - Bst-XI restriction fragment from pBS/GALR-2 was isolated and subcloned between the Hind III and BstX-I
10 sites of pcDNA3 from InVitrogen, San Diego, Ca. This expression vector, designated pCDNA3/GALR-2, contains, in addition to the entire receptor coding sequence, 50 bp of 5' untranslated sequence and 288 bp of 3' untranslated sequence. Plasmid DNA for further analysis was prepared using the Qiaprep system from Qiagen.

15 A. Transient Transfection

HEK293s cells were obtained from Cold Spring Harbor laboratory. They were maintained in culture medium at 37°C, 5% CO₂ and diluted 10 fold every 3 days. The cells were inoculated in 80 cm² flasks (2 x 10⁶ cells per flask) in Dulbecco's Modified Essential
20 Medium (DMEM, Gibco BRL), supplemented with 10% fetal bovine serum (FBS), 100 U/ml penicillin, 100 µg/ml streptomycin and 0.25 µg/ml fungizone. One day after inoculation, cells were transiently transfected using a modified CaCl₂ method (Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press (1989)) and 30 µg of plasmid DNA per flask. The cells were harvested 48 hours post transfection
25 for ligand binding or signal transduction experiments.

B. Stable Transfection

HEK293s cells in 80 cm² flasks were transfected with 30 µg pCDNA3/GALR-2. After 21
30 days of selection in culture medium containing 600 µg/ml G418 resistant colonies were pooled and expanded for the radioligand binding and signal transduction studies.

Example 4: Binding Characteristics of Recombinantly Expressed Rat GAL-R2**A. Methods**

5 A galanin binding assay was performed on the crude membranes prepared from pcDNA3/GALR-2 transfected cells. The cells were grown in 150 mm petri dishes to about 80% confluency. Before harvesting, the cells on the petri dishes were washed once with cold PBS (Gibco BRL). Cells were then scraped in ice cold PBS using a Teflon cell scraper. The cells thus harvested were gently centrifuged at 1500 x g at 4 C, resuspended in
10 membrane buffer, "MB" (20 mM HEPES pH 7.5 containing 10 µg/ml benzamidine, 5 µg/ml leupeptin, 5 µg/ml soybean trypsin inhibitor and 0.1 mM phenyl methyl sulfonyl fluoride) and were disrupted with a Polytron at a setting of ~20,000 rpm for 30 sec. The disrupted cell suspension was centrifuged at ~100,000 x g for 60 minutes at 4 C using a fixed angle rotor in a Beckman L8-70M Ultracentrifuge. The pellet thus obtained was
15 resuspended in the membrane buffer at a concentration of 1.0 - 1.5 mg/ml, aliquoted and frozen at -80 C until used.

The binding reaction was performed in a total volume of 100 µl of binding buffer (MB + 0.4% bovine serum albumin) containing 5 - 10 µg membrane protein and 0.1 nM ¹²⁵I-galanin (2200 Ci/mmol, Dupont/NEN) with or without unlabeled competitors. Non-specific binding was estimated in the presence of 1 µM of unlabeled galanin. Binding
20 reactions proceeded for 20 min at room temperature and were stopped by filtration through Unifilters-96, GF/B filters (Canberra Packard), using the 96-well Filtermate 196 filtration system from Canberra Packard. Filters were washed 5 times with 0.5 ml of ice cold 20 mM
25 HEPES pH 7.5. The filters were dried at 55 °C for one hour and then 100 µl of µScint-20 (Canberra Packard) was added per well. Filters were counted with the Topcount microplate counter from Canberra Packard.

B. Results

When transfected into HEK293 cells, pCDNA3/GALR-2 resulted in the expression of specific ^{125}I -galanin binding sites. No specific ^{125}I -galanin binding sites were generated by the transfection of the vector itself or a control pCDNA3 expression construct encoding a delta-opioid receptor. A pool of stable HEK293 cells expressing the GAL-R2 receptor was generated by selecting pCDNA3/GALR-2 transfected cells using G418 and binding experiments were performed on the membranes of these cells. An example of the results from a binding experiment is shown in figure 4.

A single class of saturable ^{125}I -galanin binding site was detected with an estimated K_d for ^{125}I -galanin of 1.68 ± 0.43 nM and a B_{max} of 1-2 pmol/mg of crude protein. Various galanin related peptides were used in competition experiments performed using ^{125}I -galanin as a tracer. The competition curves for these peptides are displayed in figure 5 and the K_i values of the peptides tested are summarized in Table 1.

Table 1: The inhibitory constants of galanin-related peptides for ^{125}I -galanin binding at GAL-R2

PEPTIDE	K_i [M]
Galanin	$2.65 \pm 0.07 \cdot 10^{-9}$
Galanin(1-16)	$1.23 \pm 0.70 \cdot 10^{-8}$
M15	$3.68 \pm 1.20 \cdot 10^{-8}$
M40	$8.30 \pm 0.49 \cdot 10^{-9}$
C7	$1.89 \pm 1.34 \cdot 10^{-7}$

20

The binding of labeled galanin was displaced by galanin and galanin related peptides but not by galanin unrelated ligands (e.g. substance P, vasoactive intestinal polypeptide, angiotensin II and dynorphin). The main difference between rat GAL-R1 and GAL-R2, however, lies in the recognition of the chimeric peptide C7, which is equipotent to galanin at the GAL-R1 receptor but is much less active at GAL-R2.

25

Example 5: Activation of cAMP

Stable pools of transfected cells were inoculated in 24 well plates and allowed to grow overnight. Before experiments, the cells were washed with PBS at 37 C and then covered with PBS containing 1 mM 3-isobutyl-1-methylxanthine (IBMX). Cells in duplicate wells were stimulated for 10 minutes at 37 C either with forskolin (0.1 mM) alone, or in the presence of various concentrations of galanin or galanin-related peptides. cAMP was extracted in ethanol, lyophilized and resuspended in 0.5 mM assay buffer. Assay of cAMP was performed using either the Biotrack cAMP Enzyme-immunoassay System (Amersham) or the Cyclic AMP [³H] Assay System (Amersham).

It was found that the activation of rat GAL-R2 in stably transfected HEK293 cells leads to a significant inhibition of forskolin-stimulated accumulation of cAMP and that this inhibition occurs in a concentration-dependent manner (figure 6). Untransfected cells failed to exhibit this effect .

Example 6: In Situ Hybridization

A. Methods

Adult male Sprague-Dawley rats (~300 gm; Charles River, St-Constant, Quebec) were sacrificed by decapitation. Brain, pituitary and spinal cord were promptly removed, snap-frozen in isopentane at -40 C for 20 s and stored at -80 C. Frozen tissue was sectioned at 14 µm in a Microm HM 500 M cryostat (Germany) and thaw-mounted onto ProbeOn Plus slides (Fisher Scientific, Montreal, Quebec). Sections were stored at -80 C prior to *in situ* hybridization.

The plasmid pCDNA3-GALR-2 was linearized using either XbaI or HindIII restriction enzymes which cut in the polylinker on either side of the inserted cDNA. Sense and antisense GAL-R2 riboprobes were transcribed *in vitro* using either T7 or SP6 RNA polymerases (Pharmacia Biotech), in the presence of [³⁵S]UTP (~800 Ci/mmol; Amersham, Oakville, Ontario). Following transcription, the DNA template was digested with DNase I (Pharmacia). Riboprobes were subsequently purified by phenol/chloro-form/isoamyl alcohol extraction and precipitated in 70% ethanol containing ammonium acetate and tRNA. The quality of labeled riboprobes was verified by polyacrylamide-urea gel electrophoresis.

Sections were postfixed in 4% paraformaldehyde (BDH, Poole, England) in 0.1 M phosphate buffer (pH 7.4) for 10 min at room temperature (RT) and rinsed in 3 changes of 2X standard sodium citrate buffer (SSC: 0.15 M NaCl, 0.015 M sodium citrate, pH 7.0).
5 Sections were then equilibrated in 0.1 M triethanolamine, treated with 0.25% acetic anhydride in triethanolamine, rinsed in 2X SSC and dehydrated in an ethanol series (50-100%). Hybridization was performed in a buffer containing 75% formamide, 600 mM NaCl, 10 mM Tris (pH 7.5), 1 mM EDTA, 1X Denhardt's solution, 50 mg/ml denatured salmon sperm DNA, 50 mg/ml yeast tRNA, 10% dextran sulfate, 20 mM dithiothreitol and
10 [³⁵S]UTP-labeled cRNA probes (10×10^6 cpm/ml) at 55 C for 18 h in humidified chambers. Following hybridization, slides were rinsed in 2X SSC at RT, treated with 20 mg/ml RNase IA in RNase buffer (10 mM Tris, 500 mM NaCl, 1 mM EDTA, pH 7.5) for 45 min at RT and washed to a final stringency of 0.1X SSC at 65 C. Sections were then
15 dehydrated and exposed to Kodak Biomax MR film for 10 days and/or dipped in Kodak NTB2 emulsion diluted 1:1 with distilled water and exposed for 3-4 weeks at 4 C prior to development and counterstaining with cresyl violet acetate. Neuroanatomical structures were identified according to the Paxinos and Watson rat brain atlas (Paxinos *et al.*, The Rat Brain in Stereotaxic Coordinates, Academic Press, N.Y. (1986)).

20

B. Results

The highest levels of rat GAL-R2 mRNA expression were observed in dorsal root ganglia with large, intermediate and small diameter cells being specifically labeled. Only diffuse labeling was observed throughout the dorsal and ventral horns of the spinal cord. In the rat
25 brain, the highest densities of GAL-R2 mRNA labeling were detected in the dorsal hippocampus, mammillary bodies and cerebellum (in particular, Purkinje cell layer). More moderate labeling was detected in the pontine nucleus as well as in a specific cranial motor nucleus. Moderate to weak hybridization was detected throughout the cerebral cortices. Other cephalic areas such as the thalamus, the remaining hypothalamus, and basal ganglia
30 were generally devoid of labeling. This distribution differs considerably from that reported for GALR-1 mRNA which is particularly well expressed in the ventral hippocampus, amygdala, supraoptic nucleus, several hypothalamic and thalamic nuclei, lateral parabrachial nucleus and locus coeruleus of rat brain.

35 The high level of GAL-R2 expression observed in dorsal root ganglia sensory neurons and more moderate levels observed in dorsal horn of the spinal cord is consistent with

galanin's role in pain transmission. The presence of high levels of GAL-R2 in dorsal hippocampus and mammillary bodies is consistent with a role in cognitive function.

Example 7: Cloning and Structural Features of Human Galanin Receptor-2

5 A human genomic DNA library prepared from human placenta (Clontech) in EMBL-3 vector was screened with a random labeled fragment (labeled with T7-Quick-Prime labeling kit cat. #27-9252-01, Pharmacia Biotech.) containing the complete coding region of rat GALR-2 cDNA. The prehybridization and hybridization conditions were as follows:

10 Prehybridization: 50% formamide, 5X Denhardt's solution, 5X SSC, 1% glycine, 100 g/ml sheared and denatured salmon sperm DNA at 42°C for 5 hours.

15 Hybridization: 50% formamide, 1X Denhardt's solution, 5X SSC, 0.3% SDS, 100 g/ml sheared and denatured salmon sperm DNA overnight at 42°C.

Wash: A wash step was performed from the low stringency of 2X SSC, 0.1% SDS at 42°C to the highest stringency of 0.2X SSC, 0.1% SDS at 60°C (65°C for Southern blots).

20 Eight positive clones were identified which were processed for secondary screening under hybridization and washing conditions identical to the first. The secondary screening resulted in identification of four clones; the other four clones were considered false-positive. The four positive clones were processed for tertiary and quaternary screening in order to obtain pure clones.

25 DNA was purified from the four pure clones discussed above and was processed for restriction analysis and Southern blot hybridization in order to identify smaller fragments which yield a positive signal. Three positively hybridizing bands (of estimated sizes ~5 kb, ~3.2 kb and ~0.7 kb) generated by the cleavage with Sac I and Rsa I restriction endonucleases (Pharmacia Biotech.) were identified by Southern blot hybridization. These
30 bands were excised from the gel and subcloned into either Sac I or Eco RV digested pBlueScript KS(-) plasmid. The plasmid constructs were subjected to sequencing by Sanger dideoxy sequencing method (T7 Sequencing kit, Pharmacia Biotech. Cat. #27-1682-01) and the ABI Prism Cycle Sequencing Kit (Cat. #402079, Perkin-Elmer) and the
35 composite sequence was constructed.

The nucleotide sequence for human GALR-2 gene is depicted in figure 2. An open reading frame of 1155 nucleotides is present putatively encoding a protein of 385 amino acids with a calculated molecular mass of 41478 kD. There is a putative intron of more than 1000 nucleotide in length after base number 420. The intronic sequence has been removed from the finalized sequence reproduced in figure 2. The exon-intron boundaries were determined based upon the consensus sequences around 5' and 3' splice sites in vertebrate pre-mRNAs (Lodish et al. Molecular Cell Biology 3rd Ed. Scientific American Books, pp 500; figure 4.) At the protein level, 84.4% amino acids are identical between rat and human GALR-2; the identity between the human GALR-2 and the rat or human GALR-1 is about 34%.

All references cited herein are fully incorporated by reference. Having now fully described the invention, it will be understood by one of skill in the art that the invention may be performed within a wide and equivalent range of conditions, parameters and the like, without affecting the spirit or scope of the invention or any embodiment thereof.

DEPOSIT OF BIOLOGICAL MATERIAL

The plasmid HUMAN GALR-2 has been deposited under the Budapest Treaty at "Deutsche Sammlung von Mikroorganismen und Zellkulturen" (DSMZ), Braunschweig, Germany. The deposit number is DSM 11632, and the date of deposit is 26 June 1997.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

5 (i) APPLICANT: Astra Pharma Inc. Canada

 (ii) TITLE OF INVENTION: A Novel Galanin Receptor

 (iii) NUMBER OF SEQUENCES: 8

10

 (iv) CORRESPONDENCE ADDRESS:

 (A) ADDRESSEE: Astra AB

 (B) STREET: S-151 85 Södertalje

 (C) CITY: Södertalje

15

 (D) STATE:

 (E) COUNTRY: Sweden

 (F) ZIP: none

 (v) COMPUTER READABLE FORM:

20

 (A) MEDIUM TYPE: Floppy disk

 (B) COMPUTER: IBM PC compatible

 (C) OPERATING SYSTEM: PC-DOS/MS-DOS

 (D) SOFTWARE: PatentIn Release #1.0, Version #1.30

25

 (vi) CURRENT APPLICATION DATA:

 (A) APPLICATION NUMBER:

 (B) FILING DATE:

 (C) CLASSIFICATION:

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 46-8 553 26000

(B) TELEFAX: 46-8 553 28820

5

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1714 base pairs

10

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

15

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

20

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CCGCGCGCAC ACCGCTCCCT CCACACCTCC AGGGGGAGTG AGCCACTCAA GTCTAAAGCA 60

GAGCGAGTCC CAGGACTTGA GCGCGGGAAG CGAATGGAGT CAGGGTCATT CGATTGCACC 120

25

TCTCTCGACT GCGGGCCGGA GCGGGGTACC ATCCTACACT CTGGGTGCTC CCTCCTCCTC 180

CCGTCCCCCG CGCACCCCTC CCCTGTCTCC TGGAGCTCGG CAGTCTCGCT GGGGCGCTGC 240

AGCGAGGGAG CAGCGTGCTC ACCAAGGACC CGGACAGCTG CGGGAGCGGC GTCCACTTTG 300

30

GTGATACCAT GAATGGCTCC GGCAGCCAGG GCGCGGAGAA CACGAGCCAG GAAGGCAGTA 360

GCGGCGGCTG GCAGCCTGAG GCGGTCCTTG TACCCCTATT TTTCGCGCTC ATCTTCCTCG 420

5 TGGGCACCGT GGGCAACGCG CTGGTGCTGG CGGTGCTGCT GCGCGGCGGC CAGGCGGTCA 480

GCACCACCAA CCTGTTCATC CTCAACCTGG GCGTGGCCGA CCTGTGTTTC ATCCTGTGCT 540

GCGTGCCTTT CCAGGCCACC ATCTACACCC TGGACGACTG GGTGTTCCGC TCGCTGCTCT 600

10 GCAAGGCTGT TCATTTCTC ATCTTTCTCA CTATGCACGC CAGCAGCTTC ACGCTGGCCG 660

CCGTCTCCCT GGACAGGTAT CTGGCCATCC GCTACCCGCT GCACTCCCGA GAGTTGCGCA 720

15 CACCTCGAAA CGCGCTGGCC GCCATCGGGC TCATCTGGGG GCTAGCACTG CTCTTCTCCG 780

GGCCCTACCT GAGCTACTAC CGTCAGTCGC AGCTGGCCAA CCTGACAGTA TGCCACCCAG 840

CATGGAGCGC ACCTCGACGT CGAGCCATGG ACCTCTGCAC CTTGCTCTTT AGCTACCTGC 900

20 TGCCAGTGCT AGTCCTCAGT CTGACCTATG CGCGTACCCT GCGCTACCTC TGGCGCACAG 960

TCGACCCGGT GACTGCAGGC TCAGGTTCCC AGCGCGCCAA ACGCAAGGTG ACACGGATGA 1020

25 TCATCATCGT GGCGGTGCTT TTCTGCCTCT GTTGGATGCC CCACCACGCG CTTATCCTCT 1080

GCGTGTGGTT TGGTCGCTTC CCGCTCACGC GTGCCACTTA CGCGTTGCGC ATCCTTTCAC 1140

ACCTAGTTTC CTATGCCAAC TCCTGTGTCA ACCCATCGT TTACGCTCTG GTCTCCAAGC 1200

30

ATTTCCGTAA AGGTTTCCGC AAAATCTGCG CGGGCCTGCT GCGCCCTGCC CCGAGGCGAG 1260

CTTCGGGCGG AGTGAGCATC CTGGCGCCTG GGAACCATAG TGGCAGCATG CTGGAACAGG 1320

5 AATCCACAGA CCTGACACAG GTGAGCGAGG CAGCCGGGCC CCTTGTCCTA CCACCCGCAC 1380

TTCCCAACTG CACAGCCTCG AGTAGAACCC TGGATCCGGC TTGTTAAAGG ACCAAAGGGC 1440

ATCTAACAGC TTCTAGACAG TGTGGCCCGA GGATCCCTGG GGGTTATGCT TGAACGTTAC 1500

10 AGGGTTGAGG CTAAAGACTG AGGATTGATT GTAGGGAACC TCCAGTTATT AAACGGTGCG 1560

GATTGCTAGA GGGTGGCATA GTCCTTCAAT CCTGGCACCC GAAAAGCAGA TGCAGGAGCA 1620

15 GGAGCAGGAG CAAAGCCAGC CATGGAGTTT GAGGCCTGCT TGAACCTACCT GAGATCCAAT 1680

AATAAAACAT TTCATATGCT CTCGTGCCGA ATTC 1714

20 (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 372 amino acids

(B) TYPE: amino acid

25 (C) STRANDEDNESS: not relevant

(D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

30 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

5

Met Asn Gly Ser Gly Ser Gln Gly Ala Glu Asn Thr Ser Gln Glu Gly
1 5 10 15

Ser Ser Gly Gly Trp Gln Pro Glu Ala Val Leu Val Pro Leu Phe Phe
10 20 25 30

Ala Leu Ile Phe Leu Val Gly Thr Val Gly Asn Ala Leu Val Leu Ala
35 40 45

Val Leu Leu Arg Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Phe Ile
15 50 55 60

Leu Asn Leu Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro
65 70 75 80

20

Phe Gln Ala Thr Ile Tyr Thr Leu Asp Asp Trp Val Phe Gly Ser Leu
85 90 95

Leu Cys Lys Ala Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser
25 100 105 110

Ser Phe Thr Leu Ala Ala Val Ser Leu Asp Arg Tyr Leu Ala Ile Arg
115 120 125

Tyr Pro Leu His Ser Arg Glu Leu Arg Thr Pro Arg Asn Ala Leu Ala
130 135 140

5 Ala Ile Gly Leu Ile Trp Gly Leu Ala Leu Leu Phe Ser Gly Pro Tyr
145 150 155 160

Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala Asn Leu Thr Val Cys His
165 170 175

10 Pro Ala Trp Ser Ala Pro Arg Arg Arg Ala Met Asp Leu Cys Thr Phe
180 185 190

Val Phe Ser Tyr Leu Leu Pro Val Leu Val Leu Ser Leu Thr Tyr Ala
15 195 200 205

Arg Thr Leu Arg Tyr Leu Trp Arg Thr Val Asp Pro Val Thr Ala Gly
210 215 220

20 Ser Gly Ser Gln Arg Ala Lys Arg Lys Val Thr Arg Met Ile Ile Ile
225 230 235 240

Val Ala Val Leu Phe Cys Leu Cys Trp Met Pro His His Ala Leu Ile
245 250 255

25 Leu Cys Val Trp Phe Gly Arg Phe Pro Leu Thr Arg Ala Thr Tyr Ala
260 265 270

Leu Arg Ile Leu Ser His Leu Val Ser Tyr Ala Asn Ser Cys Val Asn
275 280 285

30

Pro Ile Val Tyr Ala Leu Val Ser Lys His Phe Arg Lys Gly Phe Arg
290 295 300

Lys Ile Cys Ala Gly Leu Leu Arg Pro Ala Pro Arg Arg Ala Ser Gly
5 305 310 315 320

Arg Val Ser Ile Leu Ala Pro Gly Asn His Ser Gly Ser Met Leu Glu
325 330 335

Gln Glu Ser Thr Asp Leu Thr Gln Val Ser Glu Ala Ala Gly Pro Leu
10 340 345 350

Val Pro Pro Pro Ala Leu Pro Asn Cys Thr Ala Ser Ser Arg Thr Leu
355 360 365

15 Asp Pro Ala Cys
370

20 (2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1219 base pairs
(B) TYPE: nucleic acid
25 (C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

30 (iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

5
GGGTCAGCGG CACCATGAAC GTCTCGGGCT GCCCAGGGGC CGGGAACGCG AGCCAGGCGG 60
GCGGCGGGGG AGGCTGGCAC CCCGAGGCGG TCATCGTGCC CCTGCTCTTC GCGCTCATCT 120
10 TCCTCGTGGG CACCGTGGGC AACACGCTGG TGCTGGCGGT GCTGCTGCGC GCGGCCAGG 180
CGGTCAGCAC TACCAACCTG TTCATCCTTA ACCTGGGCGT GGCCGACCTG TGTTCATCC 240
TGTGCTGCGT GCCCTTCCAG GCCACCATCT ACACCCTGGA CGGCTGGGTG TTCGGCTCGC 300
15 TGCTGTGCAA GCGGGTGCAC TTCCTCATCT TCCTCACCAT GCACGCCAGC AGCTTCACGC 360
TGGCCGCCGT CTCCCTGGAC AGGTATTGG CCATCCGCTA CCCGCTGCAC TCCC GCAGC 420
20 TGCGCACGCC TCGAAACGCG CTGGCAGCCA TCGGGCTCAT CTGGGGGCTG TCGCTGCTCT 480
TCTCCGGGCC CTACCTGAGC TACTACCGCC AGTCGCAGCT GGCCAACCTG ACCGTGTGCC 540
ATCCCGCGTG GAGCGCCCCT CGCCGCCGCG CCATGGACAT CTGCACCTTC GTCTTCAGCT 600
25 ACCTGCTTCC TGTGCTGGTT CTCGGCCTGA CCTACGCGCG CACCTTGCGC TACCTCTGGC 660
GCGCCGTCTGA CCCGGTGGCC GCGGGCTCGG GTGCCCCGCG CGCCAAGCGC AAGGTGACAC 720
30 GCATGATCCT CATCGTGGCC GCGCTCTTCT GCCTCTGCTG GATGCCCCAC CACGCGCTCA 780

TCCTCTGCGT GTGGTTCGGC CAGTTCCCGC TCACGCGCGC CACTTATGCG CTTCGCATCC 840

TCTCGCACCT GGTCTCCTAC GCCAACTCCT GCGTCAACCC CATCGTTTAC GCGCTGGTCT 900

5 CCAAGCACTT CCGCAAAGGC TTCCGCACGA TCTGCGCGGG CCTGCTGGGC CGTGCCCCAG 960

GCCGAGCCTC GGGCCGTGTG TGCCTGCCG CGCGGGGCAC CCACAGTGGC AGCGTGTGG 1020

10 AGCGCGAGTC CAGCGACCTG TTGCACATGA GCGAGGCGGC GGGGGCCCTT CGTCCCTGCC 1080

CCGGCGCTTC CCAGCCATGC ATCCTCGAGC CCTGTCCTGG CCCGTCCTGG CAGGGCCCAA 1140

AGGCAGGGCA GACAGGCATT CCTGACGGTT GATGTGGCCT TGAAAGGCAC TTAGCGGGCG 1200

15 CCTGGGATGT ACAGAGTTG 1219

(2) INFORMATION FOR SEQ ID NO:4:

20

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 385 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: not relevant
- (D) TOPOLOGY: not relevant

25

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

30

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

5

Met Asn Val Ser Gly Cys Pro Gly Ala Gly Asn Ala Ser Gln Ala Gly

1 5 10 15

Gly Gly Gly Gly Trp His Pro Glu Ala Val Ile Val Pro Leu Leu Phe

10 20 25 30

Ala Leu Ile Phe Leu Val Gly Thr Val Gly Asn Thr Leu Val Leu Ala

35 40 45

15 Val Leu Leu Arg Gly Gly Gln Ala Val Ser Thr Thr Asn Leu Phe Ile

50 55 60

Leu Asn Leu Gly Val Ala Asp Leu Cys Phe Ile Leu Cys Cys Val Pro

65 70 75 80

20

Phe Gln Ala Thr Ile Tyr Thr Leu Asp Gly Trp Val Phe Gly Ser Leu

85 90 95

Leu Cys Lys Ala Val His Phe Leu Ile Phe Leu Thr Met His Ala Ser

25 100 105 110

Ser Phe Thr Leu Ala Ala Val Ser Leu Asp Arg Tyr Leu Ala Ile Arg

115 120 125

Tyr Pro Leu His Ser Arg Glu Leu Arg Thr Pro Arg Asn Ala Leu Ala
130 135 140

5 Ala Ile Gly Leu Ile Trp Gly Leu Ser Leu Leu Phe Ser Gly Pro Tyr
145 150 155 160

Leu Ser Tyr Tyr Arg Gln Ser Gln Leu Ala Asn Leu Thr Val Cys His
165 170 175

10 Pro Ala Trp Ser Ala Pro Arg Arg Arg Ala Met Asp Ile Cys Thr Phe
180 185 190

Val Phe Ser Tyr Leu Leu Pro Val Leu Val Leu Gly Leu Thr Tyr Ala
15 195 200 205

Arg Thr Leu Arg Tyr Leu Trp Arg Ala Val Asp Pro Val Ala Ala Gly
210 215 220

20 Ser Gly Ala Arg Arg Ala Lys Arg Lys Val Thr Arg Met Ile Leu Ile
225 230 235 240

Val Ala Ala Leu Phe Cys Leu Cys Trp Met Pro His His Ala Leu Ile
245 250 255

25 Leu Cys Val Trp Phe Gly Gln Phe Pro Leu Thr Arg Ala Thr Tyr Ala
260 265 270

Leu Arg Ile Leu Ser His Leu Val Ser Tyr Ala Asn Ser Cys Val Asn
30 275 280 285

Pro Ile Val Tyr Ala Leu Val Ser Lys His Phe Arg Lys Gly Phe Arg
290 295 300

5 Thr Ile Cys Ala Gly Leu Leu Gly Arg Ala Pro Gly Arg Ala Ser Gly
305 310 315 320

Arg Val Cys Ala Ala Ala Arg Gly Thr His Ser Gly Ser Val Leu Glu
325 330 335

10 Arg Glu Ser Ser Asp Leu Leu His Met Ser Glu Ala Ala Gly Ala Leu
340 345 350

Arg Pro Cys Pro Gly Ala Ser Gln Pro Cys Ile Leu Glu Pro Cys Pro
15 355 360 365

Gly Pro Ser Trp Gln Gly Pro Lys Ala Gly Gln Thr Gly Ile Pro Asp
370 375 380

20 Gly
385

(2) INFORMATION FOR SEQ ID NO:5:

25

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 345 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: not relevant

30 (D) TOPOLOGY: not relevant

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

5

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

10 Met Glu Leu Ala Pro Val Asn Leu Ser Glu Gly Asn Gly Ser Asp Pro

1 5 10 15

Glu Pro Pro Ala Glu Pro Arg Pro Leu Phe Gly Ile Gly Val Glu Asn

20 25 30

15

Phe Ile Thr Leu Val Val Phe Gly Leu Ile Phe Ala Met Gly Val Leu

35 40 45

Gly Asn Ser Leu Val Ile Thr Val Leu Ala Arg Ser Lys Pro Gly Lys

20 50 55 60

Pro Arg Ser Thr Thr Asn Leu Phe Ile Leu Asn Leu Ser Ile Ala Asp

65 70 75 80

25 Leu Ala Tyr Leu Leu Phe Cys Ile Pro Phe Gln Ala Thr Val Tyr Ala

85 90 95

Leu Pro Thr Trp Val Leu Gly Ala Phe Ile Cys Lys Phe Ile His Tyr

100 105 110

30

Phe Phe Thr Val Ser Met Leu Val Ser Ile Phe Thr Leu Ala Ala Met
115 120 125

Ser Val Asp Arg Tyr Val Ala Ile Val His Ser Arg Arg Ser Ser Ser
5 130 135 140

Leu Arg Val Ser Arg Asn Ala Leu Leu Gly Val Gly Phe Ile Trp Ala
145 150 155 160

Leu Ser Ile Ala Met Ala Ser Pro Val Ala Tyr Tyr Gln Arg Leu Phe
10 165 170 175

His Arg Asp Ser Asn Gln Thr Phe Cys Trp Glu His Trp Pro Asn Gln
180 185 190

Leu His Lys Lys Ala Tyr Val Val Cys Thr Phe Val Phe Gly Tyr Leu
15 195 200 205

Leu Pro Leu Leu Leu Ile Cys Phe Cys Tyr Ala Lys Val Leu Asn His
20 210 215 220

Leu His Lys Lys Leu Lys Asn Met Ser Lys Lys Ser Glu Ala Ser Lys
225 230 235 240

Lys Lys Thr Ala Gln Thr Val Leu Val Val Val Val Val Phe Gly Ile
25 245 250 255

Ser Trp Leu Pro His His Val Ile His Leu Trp Ala Glu Phe Gly Ala
260 265 270

30

Phe Pro Leu Thr Pro Ala Ser Phe Phe Phe Arg Ile Thr Ala His Cys

275

280

285

Leu Ala Tyr Ser Asn Ser Ser Val Asn Pro Ile Ile Tyr Ala Phe Leu

5

290

295

300

Ser Glu Asn Phe Arg Lys Ala Tyr Lys Gln Val Phe Lys Cys Arg Val

305

310

315

320

10

Asn Glu Ser Pro His Gly Asp Ala Lys Glu Lys Asn Arg Ile Asp Thr

325

330

335

Pro Pro Ser Thr Asn Cys Thr His Val

340

345

15

(2) INFORMATION FOR SEQ ID NO:6:

20

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 349 amino acids

(B) TYPE: amino acid

(C) STRANDEDNESS: not relevant

(D) TOPOLOGY: not relevant

25

(ii) MOLECULE TYPE: protein

(iii) HYPOTHETICAL: NO

30

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

	Met	Glu	Leu	Ala	Val	Gly	Asn	Leu	Ser	Glu	Gly	Asn	Ala	Ser	Cys	Pro	
5	1				5				10						15		
	Glu	Pro	Pro	Ala	Pro	Glu	Pro	Gly	Pro	Leu	Phe	Gly	Ile	Gly	Val	Glu	
				20					25						30		
10	Asn	Phe	Val	Thr	Leu	Val	Val	Phe	Gly	Leu	Ile	Phe	Ala	Leu	Gly	Val	
			35					40						45			
	Leu	Gly	Asn	Ser	Leu	Val	Ile	Thr	Val	Leu	Ala	Arg	Ser	Lys	Pro	Gly	
			50				55							60			
15	Lys	Pro	Arg	Ser	Thr	Thr	Asn	Leu	Phe	Ile	Leu	Asn	Leu	Ser	Ile	Ala	
			65				70				75				80		
	Asp	Leu	Ala	Tyr	Leu	Leu	Phe	Cys	Ile	Pro	Phe	Gln	Ala	Thr	Val	Tyr	
20					85					90					95		
	Ala	Leu	Pro	Thr	Trp	Val	Leu	Gly	Ala	Phe	Ile	Cys	Lys	Phe	Ile	His	
					100				105						110		
25	Tyr	Phe	Phe	Thr	Val	Ser	Met	Leu	Val	Ser	Ile	Phe	Thr	Leu	Ala	Ala	
					115				120						125		
	Met	Ser	Val	Asp	Arg	Tyr	Val	Ala	Ile	Val	His	Ser	Arg	Arg	Ser	Ser	
					130				135						140		
30																	

Ser Leu Arg Val Ser Arg Asn Ala Leu Leu Gly Val Gly Cys Ile Trp
145 150 155 160

Ala Leu Ser Ile Ala Met Ala Ser Pro Val Ala Tyr His Gln Gly Leu
5 165 170 175

Phe His Pro Arg Ala Ser Asn Gln Thr Phe Cys Trp Glu Gln Trp Pro
180 185 190

Asp Pro Arg His Lys Lys Ala Tyr Val Val Cys Thr Phe Val Phe Gly
10 195 200 205

Tyr Leu Leu Pro Leu Leu Leu Ile Cys Phe Cys Tyr Ala Lys Val Leu
210 215 220

Asn His Leu His Lys Lys Leu Lys Asn Met Ser Lys Lys Ser Glu Ala
15 225 230 235 240

Ser Lys Lys Lys Thr Ala Gln Thr Val Leu Val Val Val Val Val Phe
20 245 250 255

Gly Ile Ser Trp Leu Pro His His Ile Ile His Leu Trp Ala Glu Phe
260 265 270

Gly Val Phe Pro Leu Thr Pro Ala Ser Phe Leu Phe Arg Ile Thr Ala
25 275 280 285

His Cys Leu Ala Tyr Ser Asn Ser Ser Val Asn Pro Ile Ile Tyr Ala
290 295 300

Phe Leu Ser Glu Asn Phe Arg Lys Ala Tyr Lys Gln Val Phe Lys Cys

305 310 315 320

His Ile Arg Lys Asp Ser His Leu Ser Asp Thr Lys Glu Asn Lys Ser

5 325 330 335

Arg Ile Asp Thr Pro Pro Ser Thr Asn Cys Thr His Val

340 345

10

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 37 base pairs

15 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

20 (A) DESCRIPTION: /desc = "PCR primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GGCCGTCGAC TTCATCGTCW MYCTIKCIYT IGCNGAC 37

30

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs

5 (B) TYPE: nucleic acid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: other nucleic acid

10 (A) DESCRIPTION: /desc = "PCR primer"

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

15


(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

RHWRCARTAI ATATIGGRT T 21

INDICATIONS RELATING TO A DEPOSITED MICROORGANISM

(PCT Rule 13bis)

A. The indications made below relate to the microorganism referred to in the description on page <u>23</u> , line <u>21</u>	
B. IDENTIFICATION OF DEPOSIT Further deposits are identified on an additional sheet: <input type="checkbox"/>	
Name of depositary institution Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ)	
Address of depositary institution (including postal code and country) Mascheroder Weg 1b D-38124 Braunschweig Germany	
Date of deposit 26 June 1997	Accession Number DSM 11632
C. ADDITIONAL INDICATIONS (leave blank if not applicable) This information is continued on an additional sheet: <input type="checkbox"/>	
In respect of all designated states in which such action is possible and to the extent that it is legally permissible under the law of the designated state, it is requested that a sample of the deposited micro-organism be made available only by the issue thereof to an independent expert, in accordance with the relevant patent legislation, e.g. Rule 28(4) EPC, and generally similar provisions <i>mutatis mutandis</i> for any other designated state.	
D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g. "Accession Number of Deposit")	

<div style="text-align: right; font-size: small;">For receiving Office use only</div> <div style="padding: 5px;"><input checked="" type="checkbox"/> This sheet was received with the international application</div> <div style="padding: 5px;">Authorized officer </div>	<div style="text-align: right; font-size: small;">For International Bureau use only</div> <div style="padding: 5px;"><input type="checkbox"/> This sheet was received by the International Bureau on:</div> <div style="padding: 5px;">Authorized officer</div>
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Claims

1. A protein, except as existing in nature, comprising the amino acid sequence consisting functionally of rat GAL-R2 as shown in SEQ ID NO:2.
2. A substantially pure protein according to claim 1, wherein said amino acid sequence consists essentially of the amino acid sequence of SEQ ID NO:2.
3. An antibody made by a process comprising the step of injecting a pharmaceutically acceptable preparation comprising the protein of either claim 1 or claim 2 into an animal capable of producing said antibody.
4. An antibody that binds specifically to the protein of claim 2.
5. A substantially pure polynucleotide encoding a protein comprising the amino acid sequence consisting functionally of rat GAL-R2 as shown in SEQ ID NO:2.
6. The polynucleotide of claim 5, wherein said polynucleotide encodes a protein consisting essentially of the amino acid sequence of SEQ ID NO:2.
7. A vector for expressing GAL-R2, comprising the polynucleotide of either claim 5 or claim 6.
8. A host cell transformed with the vector of claim 7.
9. Recombinant GAL-R2 produced by the host cell of claim 8.
10. The polynucleotide of claim 6, wherein said polynucleotide has the nucleotide sequence of SEQ ID NO:1.
11. A vector for expressing GAL-R2 comprising the polynucleotide of claim 10.
12. A host cell transformed with the vector of claim 11.
13. A protein, except as existing in nature, comprising the amino acid sequence consisting functionally of human GAL-R2 as shown in SEQ ID NO:4.

14. A substantially pure protein according to claim 13, wherein said amino acid sequence consists essentially of the amino acid sequence of SEQ ID NO:4.
- 5 15. An antibody made by a process comprising the step of injecting a pharmaceutically acceptable preparation comprising the protein of either claim 13 or claim 14 into an animal capable of producing said antibody.
16. An antibody that binds specifically to the protein of claim 14.
- 10 17. A substantially pure polynucleotide encoding a protein comprising the amino acid sequence consisting functionally of human GAL-R2 as shown in SEQ ID NO:4.
18. The polynucleotide of claim 17, wherein said polynucleotide encodes a protein
15 consisting essentially of the amino acid sequence of SEQ ID NO:4.
19. A vector for expressing GAL-R2, comprising the polynucleotide of either claim 17 or claim 18.
- 20 20. A host cell transformed with the vector of claim 19.
21. Recombinant GAL-R2 produced by the host cell of claim 20.
22. The polynucleotide of claim 18, wherein said polynucleotide has the nucleotide
25 sequence of SEQ ID NO:3.
23. A vector for expressing GAL-R2 comprising the polynucleotide of claim 22.
24. A host cell transformed with the vector of claim 23.

25. A method for assaying a test compound for its ability to bind or to activate GAL-R2, comprising:

- 5 a) incubating a source containing GAL-R2 but substantially free of other galanin receptors, with;
i) a ligand known to bind to GAL-R2;
ii) said test compound; and
- 10 b) determining the extent to which said ligand binding is displaced by said test compound.

26. The method of claim 25, wherein said source of GAL-R2 is a cell transformed with a vector for expressing rat GAL-R2 and comprising a polynucleotide encoding a protein
15 consisting essentially of the amino acid sequence of SEQ ID NO:2.

27. The method of claim 25, wherein said source of GAL-R2 is a membrane preparation derived from a cell transformed with a vector for expressing rat GAL-R2 and comprising a polynucleotide encoding a protein consisting essentially of the amino
20 acid sequence of SEQ ID NO:2.

28. The method of claim 25, wherein said source of GAL-R2 is a cell transformed with a vector for expressing human GAL-R2 and comprising a polynucleotide encoding a protein consisting essentially of the amino acid sequence of SEQ ID NO:4.
25

29. The method of claim 25, wherein said source of GAL-R2 is a membrane preparation derived from a cell transformed with a vector for expressing human GAL-R2 and comprising a polynucleotide encoding a protein consisting essentially of the amino
30 acid sequence of SEQ ID NO:4.

30. The method of claim 25, further comprising the step of determining activation of a second messenger pathway such as the adenylyl cyclase activity in cells.

31. A method for assaying a test compound for its ability to alter the expression of GAL-R2, comprising:

- (a) growing cells expressing GAL-R2 but substantially free of other galanin receptors in the presence of said test compound;
- (b) collecting said cells; and
- (c) comparing receptor expression in the cells exposed to said test compound with control cells grown under essentially identical conditions but not exposed to said test compound.

32. The method of claim 31, wherein said cells expressing GAL-R2 are cells transformed with an expression vector comprising a polynucleotide sequence encoding a protein with an amino acid sequence consisting essentially of the sequence of rat GAL-R2 as shown in SEQ ID NO:2.

33. The method of claim 32, wherein said test compound is an oligonucleotide at least 15 nucleotides in length and comprising a sequence complementary to a sequence shown in SEQ ID NO:2.

34. The method of claim 31, wherein said cells expressing GAL-R2 are cells transformed with an expression vector comprising a polynucleotide sequence encoding a protein with an amino acid sequence consisting essentially of the sequence of human GAL-R2 as shown in SEQ ID NO:4.

35. The method of claim 34, wherein said test compound is an oligonucleotide at least 15 nucleotides in length and comprising a sequence complementary to a sequence shown in SEQ ID NO:4.

36. The method of claim 31, wherein said receptor expression is determined by means of receptor binding assays.

25 * 50 75 100
 CCGCGGCACACCGCTCCCTCCACA CCTCCAGGGGAGTGAGCCACICTAA GTCTAAGCAGAGCGAGTCCACGGA CTTGAGCGCGGGAAGCGAATGGAGT *

125 * 150 175 200
 CAGGTCATTCGATTGCACCTCTCT CCACTGCGGGCGGAGCGGGTACC ATCTACACICTGGGIGCTCCCTCC TCCTCCCGTCCCGCGGACCCCTC *

225 * 250 275 300
 CCCIGTCTCCAGGTCGGCAGTC TCGCTGGGGCGCTCCAGCGAGGAG CAGCGTGGTACCAAGGACCGGAC AGCTCGGGAGCGGCTCCACTTTG *

325 * 350 375
 GTGATACC ATG AAT GGC TCC GGC AGC CAG GGC GGC GAG AAC ACC AGC CAG GAA GGC AGT ACC GGC GGC TGG CAG CCT GAG *
 M N G S G S Q G A E N T S Q E G S S G G W Q P E

400 * 425 450
 GCG GTC CTT GTA CCC CTA TTT TTC GCG CTC ATC TTC CTC GTG GGC ACC GTG GGC AAC GCG CTC GTG CTC GCG GTG CTG *
 A V L V P L F F A L I F L V G T V G N A L V L A V L

475 * 500 525
 CTG CGC GGC GGC CAG CCG GTC AGC ACC AAC CTG TTC ATC CTC AAC CTG GGC GTG GGC GAC CTG TGT TTC ATC CTG *
 L R G G Q A V S T T N L F I L N L G V A D L C F I L

FIG.1A

2/ 11

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550          575          600
TGC TGC GTG CCT TTC CAG GCC ACC ATC TAC ACC CTG GAC GAC TGG GTG TTC GGC TCG CTG CTC TGC AAG GCT GTT CAT
C C V P F Q A T I Y T L D D W V F G S L L C K A V H

625          650          675
TTC CTC ATC TTT CTC ACT ATG CAC GCC AGC AGC TTC AGG CTG GCC GGC GTG TCC CTG GAC AGG TAT CTG GCC ATC CGC
F L I F L T M H A S S F T L A A V S L D R Y L A I R

700          725          750
TAC CCG CTG CAC TCC CGA GAG TTG CGC ACA CCT CGA AAC GCG CTG GCC GGC ATC GCG CTC ATC TGG GGG CTA GCA CTG
Y P L H S R E L R T P R N A L A A I G L I W G L A L

775          800          825
CTC TTC TCC GGG CCC TAC CTG AGC TAC TAC CGT CAG TCG CAG CTG GCC AAC CTG ACA GTA TGC CAC CCA GCA TGG AGC
L F S G P Y L S Y Y R Q S Q L A N L T V C H P A W S

850          875          900          925
GCA CCT CGA CGT CGA GCC ATG GAC CTC TGC ACC TTC CTC TTT AGC TAC CTG CTG CCA GTG CTA GTC CTC AGT CTG ACC
A P R R R A M D L C T F V F S Y L L P V L V L S L T

```

FIG.1B

```

950          975          1000
TAT GCG CGT ACC CIG CGC TAC CTC TGG CGC ACA GTC GAC CCG GIG ACT GCA GGC TCA GGT TCC CAG CGC GCC AAA CGC
Y A R T L R Y L W R T V D P V T A G S G S Q R A K R

1025          1050          1075
AAG GTG ACA CGG ATG ATC ATC GTG GCG GTG CTT TTC TGC CTC TGT TGG ATG CCC CAC CAC GCG CTT ATC CTC TGC
K V T R M I I I V A V L F C L C W M P H A L I L C

1100          1125          1150
GIG TGG TTT GGT CGC TTC CCG CTC ACG CGT GCC ACT TAC GCG TTG CCG ATC CTT TCA CAC CTA GTT TCC TAT GCC AAC
V W F G R F P L T R A T Y A L R I L S H L V S Y A N

1175          1200          1225
TCC TGT GTC AAC CCC ATC GTT TAC GCT CTG GTC TCC AAG CAT TTC CGT AAA GGT TTC CGC AAA ATC TGC GCG GGC CTG
S C V N P I V Y A L V S K H F R K G F R K I C A G L

1250          1275          1300
CTG CGC CCT GCC CCG AGG CGA GCT TCG GCG CGA GTG AGC ATC CTG GCG CCT GCG AAC CAT AGT GGC AGC ATG CTG GAA
L R P A P R R A S G R V S I L A P G N H S G S M L E

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FIG.1C

4/11

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1325      *      1350      *      1375
CAG GAA TCC ACA CAC CTG ACA CAG GTG ACC GAG GCA GCC GGG CCC CTT GTG CCA CCA CCC GCA CTT CCC AAC TGC ACA
Q E S T D L T Q V S E A A G P L V P P P A L P N C T

1400      *      1425      *      1450      *      1475
GCC TCG AGT AGA ACC CTG GAT CCG GCT TGT T AAAGGACCAAGGCCATCTAACAGC TTCTAGACAGTGTGGCCCGAGGATC
A S S R T L D P A C

1500      *      1525      *      1550      *      1575
CCTGGGGTTATGCTTGAACGTTAC AGGTTGAGGCTAAAGACATGAGGAT TCATTGTAGGGAACCTCCAGTTATT AAACGGTGGGATTGCTAGAGGGTG

1600      *      1625      *      1650      *      1675
GCATAGTCCTTCAATCCIGGCACCC GAAAAGCAGATGCAGGAGCAGGAGC AGGAGCAAGCCAGCCATGGAGTTT GAGGCCCTGCTTGAACCTACCTGAGAT

1700      *
CCAATAATAAACAATTTCATATGCT CTCGTGCCGAATTC

```

FIG.1D

* * * * *
 GCGTCAGCGGACC ATG AAC GTC TCG GGC TGC CCA GGC GGC GGC AAC GCG ACC CAG GCG GGC GGC GGA GGC TGG CAC CCC GAG GCG GTC ATC GTC CCC CTC CTC TTC GCG
 M N V S C G C P G A G N A S Q A C G C G C G W H P E A V I V P L L F A

120 * * * * * 150 180 210
 CTC ATC TTC CTC GTC GGC ACC GTC GGC AAC ACC GTC GTC GTC GGC GTC GGC GGC GGC CAG GGC GTC AGC ACT ACC AAC CTC TTC ATC CTT AAC CTC GCG CTC GCG
 L I F L V G T V G N T L V L A V L L R G G Q A V S T T N L F I L N L G V A

240 * * * * * 270 300 330
 GAC CTG IGT TTC ATC CTG TGC TGC GTC CCC TTC CAG GGC ACC ATC TAC ACC CTG GAC GGC TGC GTC TGC GGC TGC GGC GGC GTC CAC TTC CTC ATC TTC CTC
 D L C F I L C C V P F Q A T I Y T L D G W V F G S L L C K A V H F L I F L

360 * * * * * 390 420
 ACC ATG CAC GCG ACC AGC TTC ACC CTG GCG GGC GTC TCC CTG GAC AGG TAT TTG GCG ATC GCG TAC CCG CTG CAC TCC GCG GAG CTG CCG AGC CCT CGA AAC GCG CTC GCA
 T M H A S S F T L A A V S L D R Y L A I R Y P L H S R E L R T P R N A L A

FIG.2A

450 * * * * * 510 540
 GCC ATC GCG CTC ATC TGG GCG CTC TCG CTC CTC TAC GCG CAG TCG CAG CTC GCG AAC CTC ACC GTC TGC CAT CCC GCG TGG AGC GCC
 A I G L I W G L S L L F S G P Y L S Y Y R Q S Q L A N L I V C H P A W S A
 570 600 630 660
 CCT CCG CCG CCG ATG GAC ATC TGC ACC TTC GTC TTC ACC TAC CTC CTT CTT GTC GTC GTC GCG CCG ACC TIG CCG TAC CTC TGG CCG GCC GTC
 P R R A M D I C T F V F S Y L L P V L V L G L T Y A R T L R Y L W R A V
 690 720 750
 GAC CCG GTG CCG CCG GCG TCG GGT GCG CCG CCG CCG AAC GCG AAC GTC ACA CCG ATG ATC CTC ATC GTC GCG CCG CTC TTC TGC CTC TGC TGC ATG CCC CAC GCG CTC
 D P V A A G S G A R R A K R K V T R M I L I V A A L F C L C W M P H A L

FIG.2B

```

780      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      ATC CTC TGC GTG TGG TTC GGC CAG TTC CCG CTC ACG GGC GGC ACT TAT GCG CTT CCG ATC CTC TCG CAC CTG GTC TCC TAC GGC AAC TCC TGC GTC AAC CCC ATC GTT TAC
      I L C V W F G Q F P L T R A T Y A L R I L S H L V S Y A N S C V N P I V Y

810      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      840      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      870      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *

900      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      GCG CTG GTC TCC AAG CAC TTC CCG AAA GGC TTC CCG ACG ATC TGC GCG CTC GGC CCG CTT GGC CGA GGC TCG GGC CGT GTG TGC GCT GGC GCG GCG ACC
      A L V S K H F R K G F R T I C A G L L G R A P G R A S G R V C A A A R G T

930      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      960      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      990      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *

1020      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      CAC AGT GCG ACG GTG TTG GAG CCG GAG TCC ACG CAC CTG TTG CAC ATG ACG GAG GCG GCG GCG CTT CGT CCG TCG CCC GGC GCT TCC CAG CCA TGC AIC CTC GAG CCC
      H S G S V L E R E S S D L L L H M S E A A G A L R P C P G A S Q P C I L E P

1050      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      1080      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      1110      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *

1140      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      1170      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *
      1200      *      *      *      *      *      *      *      *      *      *      *      *      *      *      *

      TGT CCT GCG CCG TCC TGG CAG GCG CCA AAG CCA GCG CAG ACA GCG ATT CCI CAC GGT TCA TGTGCCCCI TGAAGGCACATTAGCGGCG CCTGGCATGTACAGATTG
      C P G P S W Q G P K A G Q T G I P D G

```

FIG.2C

8/11

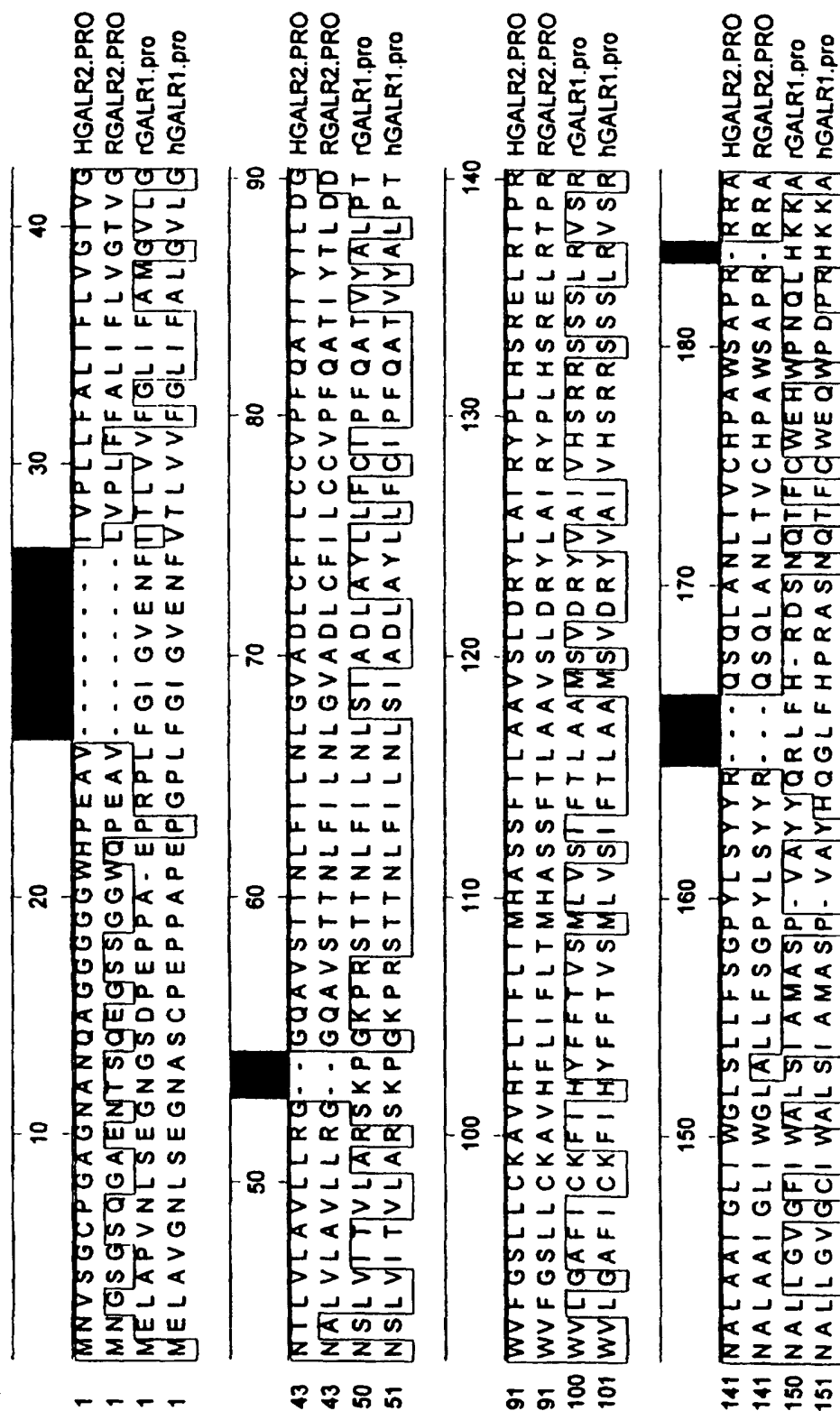
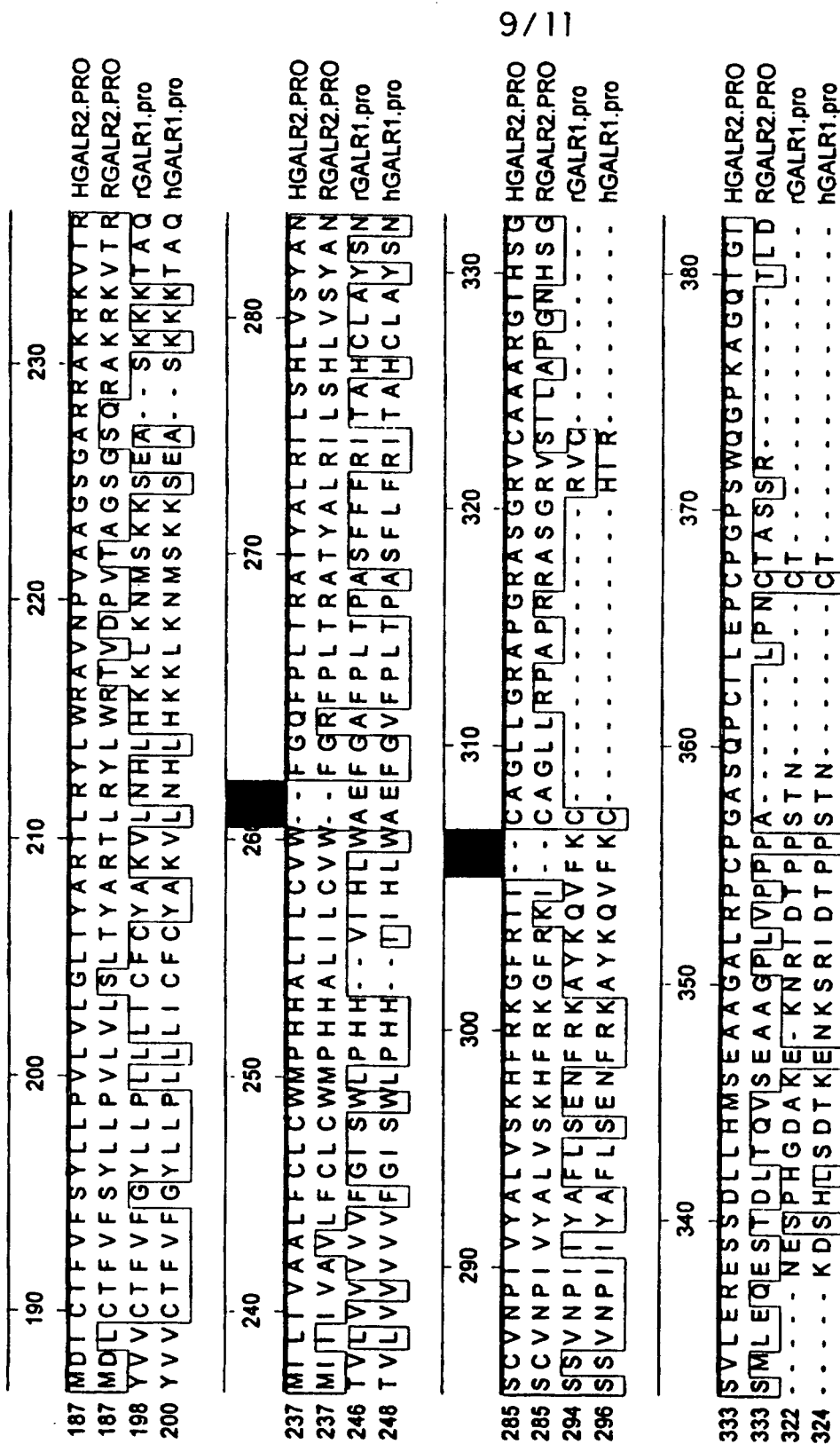


FIG.3A



HGALR2.PRO
 RGALR2.PRO
 rGALR1.pro
 hGALR1.pro

FIG. 3B

383 PDG
 370 PAC
 345 HV.
 348 HV.

10/11

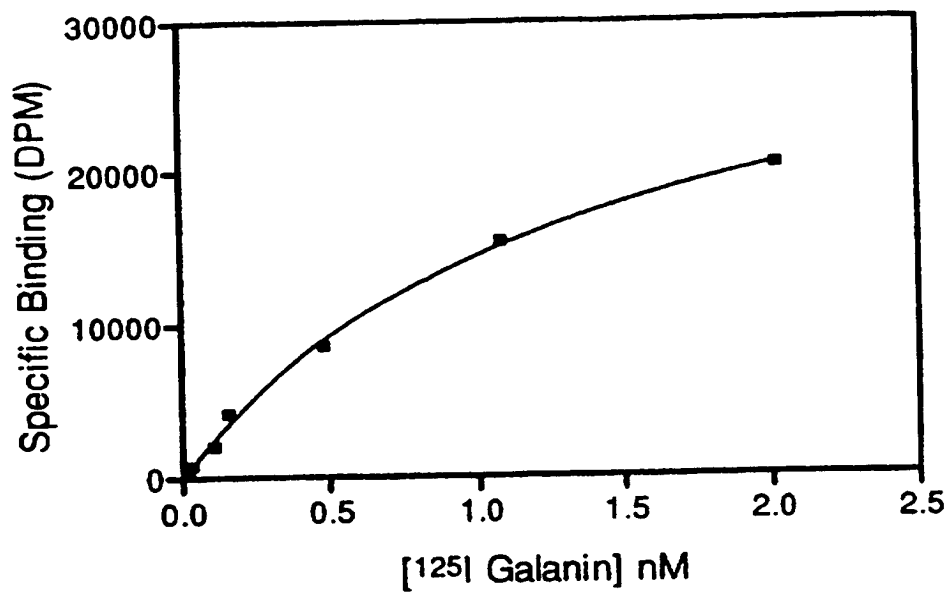


FIG. 4

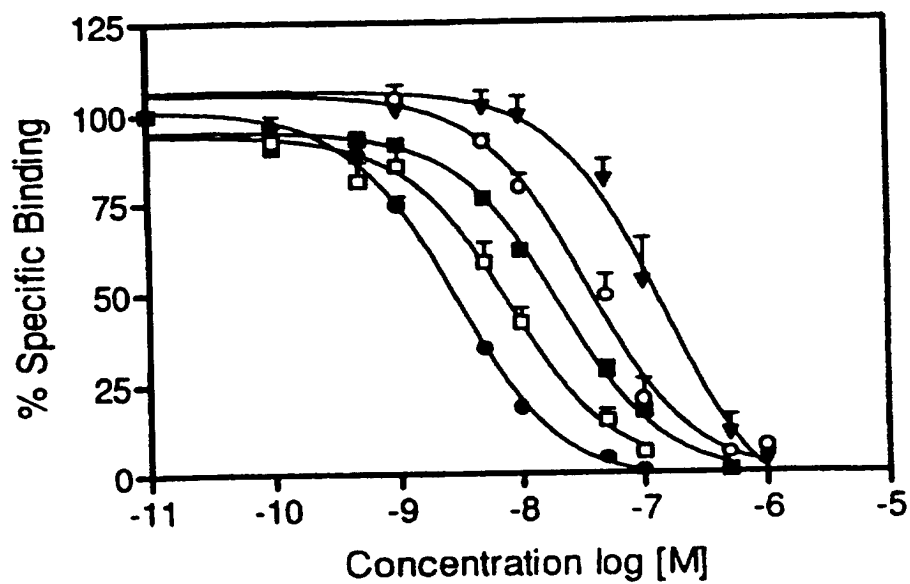


FIG. 5

11/11

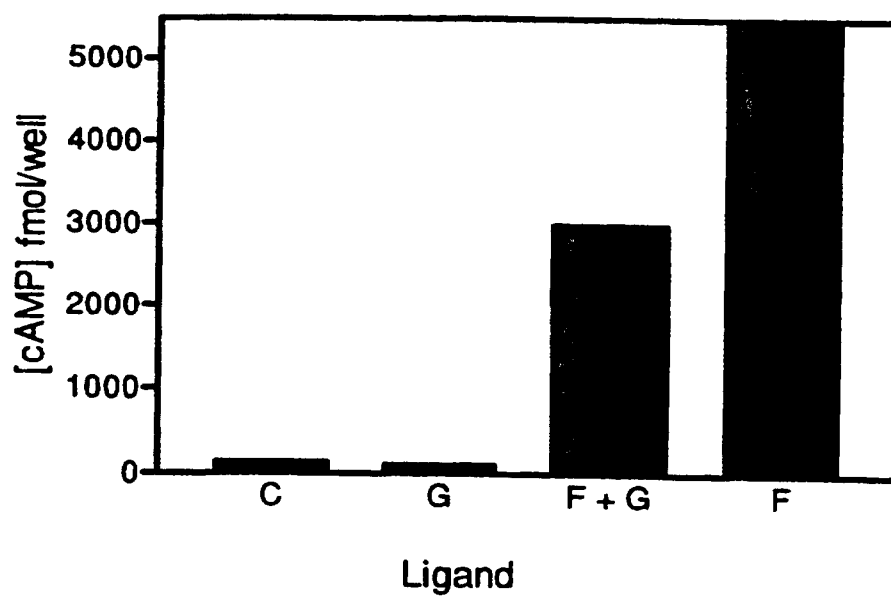


FIG. 6A

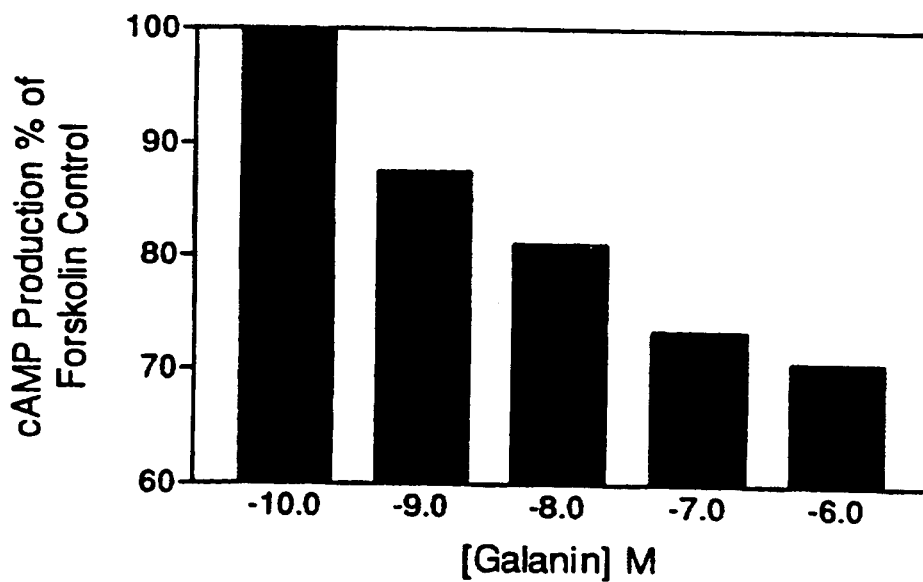


FIG. 6B

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 97/01217

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 14/72, C07K 16/28, C12N 15/11
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: C07K, C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

WPI, MEDLINE, BIOSIS, DBA, GENBANK/EMBL/SWISSPROT/DBJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 9215015 A1 (ZYMOGENETIC, INC.), 3 Sept 1992 (03.09.92), claims 1-2	25-30
A	--	1-24,31-36
A	EP 0711830 A2 (TAKEDA CHEMICAL INDUSTRIES, LTD.), 15 May 1996 (15.05.96), page 8, line 55 - page 9, line 2, claims	1-36
A	WO 9522608 A1 (RHONE-POULENC RORER S.A.), 24 August 1995 (24.08.95)	1-36

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:

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Date of the actual completion of the international search

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Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

Information on patent family members

01/10/97

International application No.

PCT/SE 97/01217

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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EP 0711830 A2	15/05/96	CA 2160449 A	14/04/96
		JP 9048800 A	18/02/97
WO 9522608 A1	24/08/95	AU 1814995 A	04/09/95
		CA 2182621 A	24/08/95
		EP 0745122 A	04/12/96
		FR 2716205 A,B	18/08/95